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# CEREAL CHEMISTRY

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No. 1

## COMMERCIAL PRODUCTION AND USE OF MOLD BRAN

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(Presented at the Annual Meeting, May 1946; received for publication July 29, 1946)

The purpose of this paper is to present some of the laboratory, pilot plant, and semicommercial investigations which have led to the successful commercial production of mold bran, a primary fungal amylase preparation. The pioneering research was conducted by the authors and their collaborators in four laboratories, Iowa State College, University of Idaho, University of Nebraska, and Farm Crops Processing Corporation, over a period of nearly 10 years. Space permits presentation here of only the most salient points.

The use of amylases in industrial processes has long been of interest to cereal chemists. Although barley malt amylases have been most generally used in industry, amylase concentrates prepared from fungal or bacterial sources have also found limited use in a number of industrial processes. The amylases from various sources differ in properties, such as extent of liquefaction, dextrinization and saccharification of starch, temperature for optimum activity, thermal stability, and optimum pH range. These differences in properties have occasioned much interest in recent years and investigations have been made to determine more accurately the special properties of cereal, bacterial, and fungal amylases in order to be able to select or adapt the most favorable for specific industrial uses. Research has also been active in an effort to develop more efficient and cheaper methods for the production of bacterial and fungal amylase preparations of high activity.

The largest industrial use of amylases is for the saccharification of starch for the production of alcohol by fermentation of grains and potatoes. For alcoholic fermentation of starchy substrates in occidental countries, barley malt has most generally been used as the sac-

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charifying agent, whereas in the Orient fungal amylases have been most extensively employed. Suggestions have frequently been made that fungal amylases be substituted for malt in the alcoholic fermentation of grains in America and in Europe, but developments along this line have not been significant, particularly in America. In Europe the *amyl* process, which uses fungal amylases produced by growing selected molds in the grain mash prior to yeast fermentation, has been employed to a limited extent. Grove (1914) and Owen (1933) have described this process in some detail.

Takamine (1914) first suggested the use of a product prepared by growing the mold *Aspergillus oryzae* on wheat bran to replace malt in alcoholic fermentation of grains, but without significant industrial developments. This idea was recently revived by Underkofler, Fulmer, and Schoene (1939), and favorable results in the use of such fungal preparations, designated as mold bran, have been reported by these authors, by Hao, Fulmer, and Underkofler (1943), by Roberts, Laufer, Stewart, and Saletan (1944), and by Hao and Jump (1945) on the laboratory scale. Beresford and Christensen (1941) reported the successful use of mold bran in the experimental alcohol plant of the University of Idaho, yields of alcohol from potatoes being somewhat better with mold bran than with malt. Recently Underkofler, Severson, and Goering (1946) reported extensive commercial tests in which yields of alcohol were at least as good or somewhat better with mold bran as with malt.

In order for mold bran to be accepted in the alcohol fermentation industry, or in other industries employing amylases, it must possess high amylolytic activity and be readily available at lower relative cost than barley malt. That is, a cheap and efficient method of commercial production is required. The research reported in this paper has resulted in such successful commercial application.

### Laboratory Investigations

Takamine (1913, 1914, 1918) recommended the use of a rotating drum for the production of mold bran on an industrial scale. In his process the bran was steamed, inoculated with spores of the mold *Aspergillus oryzae*, and incubated in a slowly rotating drum through which humidified air was passed. Takamine experienced considerable difficulty with contamination by undesirable microorganisms, especially bacteria. In an effort to minimize such contamination he (1913a, 1914, 1915) attempted to acclimatize the mold culture to concentrations of formaldehyde sufficient to prevent growth of the undesirable contaminants.

During the early laboratory work of the authors and their co-

workers, the drum method of Takamine was employed, and has been described in detail by Underkofler, Fulmer, and Schoene (1939). It was found during the course of this early work that use of dilute hydrochloric acid, rather than water, to moisten the bran gave better results. By acidifying the bran with 0.1 to 0.3 *N* hydrochloric acid to a pH of 3.5 to 4.5, sterilization was improved, and the presence of the acid inhibited the growth of undesirable microorganisms during the growth period of the mold. Underkofler, Fulmer, and Schoene (1939) described this development, and the use of acid for this purpose was patented by Underkofler (1942) and by Christensen (1944).

In going from the 5-gallon laboratory drums to larger drums, difficulty was experienced in securing good growth of the mold, owing to destruction of the delicate mold mycelium in the early growth stages by the tumbling of the bran particles. The larger the drum, the greater was the difficulty experienced. Hence, one of the authors, at the University of Idaho, developed a laboratory method involving the incubation of the mold in a small covered pan having a perforated bottom permitting air to be forced through the bran mass. A modification of this procedure was described in detail by Hao, Fulmer, and Underkofler (1943), and this method has been employed in most of the laboratory work. In this procedure the bran is moistened, sterilized, inoculated with mold spores, and held in the covered pan. Air is passed through the bran in the pan at such rate that the temperature is kept at 35° to 40°C, the direction of aeration being reversed at hourly intervals by alternately applying pressure and suction. After 24 hours the contents are removed from the pan, broken into pieces 1 inch in diameter or less, placed on a table in a pile about 2 inches deep, and covered with a damp cloth. It is normally held for another 24 hours, and is then dried at room temperature.

During the course of the work at the University of Idaho it was found desirable to add to the bran traces of salts of iron, zinc, and copper and of phosphates. At Iowa State College it was found that with some wheat brans the addition of these materials had little effect on the rate or extent of the growth of *A. oryzae* or on the amylolytic activity of the resulting mold bran. On the other hand, with certain brans addition of these materials resulted in considerably better mold growth and higher amylolytic activity. The use of such mineral salts and phosphates was patented by Christensen (1944).

Hao, Fulmer, and Underkofler (1943) investigated the saccharifying ability, for alcoholic fermentation mashes, of mold bran preparations produced by 27 different strains of molds. Several strains of the species *A. oryzae*, *Rhizopus delemar*, and *R. oryzae* gave products which were almost equal in saccharifying ability as measured by fer-

mentation tests on corn mashes. It was concluded, however, that strains of *A. oryzae* were most satisfactory because of superior cultural characteristics, including more abundant sporulation and denser mycelial growth.

Another method which was tested on the laboratory scale for the preparation of mold bran was incubation on trays. Excellent growth of the mold resulted in such tests conducted at the University of Idaho and the University of Nebraska. The bran was simply mixed with dilute acid, sterilized, cooled at about 35°C, inoculated with spores of *A. oryzae*, placed on the trays in thin layers, and incubated at 30° to 35°C in cabinets in which humidified air was circulated. The tray method was employed on a pilot plant scale at the experimental alcohol plant of the University of Idaho for the production of mold bran in 100-pound lots.

The mold bran produced by any of the laboratory procedures was dried by spreading it out on the table top and allowing it to air-dry at room temperature. If piled in thick layers, or if allowed to remain in chunks larger than about one inch in diameter, the moist material heats rapidly, resulting in autolysis of the mold and destruction of the enzymes present. It has been found that the amylase potency for saccharifying fermentation mashes does not decrease to a measurable extent even after storage of the dry mold bran for periods up to 24 months. The critical moisture level for storage without deterioration is about 15%. It was found in the laboratory that the mold bran can be used equally well for saccharifying grain mashes either in the moist condition directly after production or after drying the material. In either case it is advisable to prepare a slurry of the mold bran in water before use. There is no advantage in grinding the mold bran to reduce the particle size.

Throughout the research there has been a continued effort to find a rapid laboratory test that would serve to evaluate the mold bran as well as other saccharifying agents, that is, a method to indicate the amount of saccharifying agent required and the alcohol yield that could be expected. However, no method except actual fermentation tests on a series of mashes with several levels of the saccharification agent has given satisfactory results. Thorne, Emerson, Olson, and Peterson (1945) came to a similar conclusion with respect to the evaluation of malts for alcohol production, and Kneen (1945) has emphasized the deficiency of customary methods for evaluating starch-degrading properties for fermentation purposes. Hence, a fermentation procedure was used for the final evaluation of all the mold bran preparations. Standardized conditions were used, and the results were controlled by comparison with fermentations of mashes saccharified with malt.

A great deal of research was required to develop a satisfactory method for growing spore cultures on bran to serve as inoculum. It was found that if, during the incubation period, the bran dries too rapidly, poor spore production is obtained; if the bran does not dry rapidly enough after sporulation is complete, autolysis occurs, with a rapid loss in spore viability. A good dry spore culture of *A. oryzae* is a greenish color, not brown or black, and a dense cloud of spores arises when the flask containing it is shaken. Spore cultures are best prepared by mixing materials in the proportions of 10 g ground corn, 100 g wheat bran, and 60 ml 0.2 *N* hydrochloric acid containing 0.62 ppm  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.63 ppm  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.08 ppm  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , distributing the moist material in 10-g quantities in 250-ml flasks and sterilizing in the autoclave. The cooled bran is then inoculated with the mold spores, the bran distributed on one side of the flask by gentle tapping, and the flask incubated at 30°C while lying on the side. The presence of corn meal and salts results in more rapid growth and sporulation of the mold and tends to control the rate of drying so that well-sporulated cultures uniformly result. In the laboratory, about 1% of dry spore culture was used in inoculating the moist, sterile bran for producing mold bran.

### Pilot Plant Investigations

During the early war period when the shortage of malt began to appear, a pilot plant research project was established by the Office for Production Research and Development of the War Production Board for the purpose of developing feasible methods for the commercial production of mold bran. In the laboratory investigations a number of problems had been encountered, the solution of which was imperative in order to translate the laboratory methods to the plant scale. The principal problems were the following: (1) methods for handling the bulky bran, for mixing it with dilute acid, and for cooking and sterilizing it; (2) methods for growing spore cultures for inoculum in large amounts; (3) methods for inoculation and the proportion of inoculum to be employed; (4) methods for incubation during the growing period; (5) methods for usage and storage of the finished mold bran. The pilot plant research is discussed below under these headings.

1. *Mixing, Cooking, and Sterilizing.* For large-scale production of mold bran an efficient system for mixing the bran with the dilute acid and for cooking the bran was needed. Pressure sterilization of large masses of wet bran in bulk presents serious problems. It was believed that the most feasible method for cooking to insure thorough sterilization of the bran was by direct steam injection in a unit which provided

for continuous agitation so that the moist bran particles would be in constant direct contact with the steam. Experience during the pilot plant operations in the experimental alcohol plant of the University of Idaho had shown that when acid was employed for moistening the bran it was only necessary to hold the bran at 93° to 99°C for 15 to 30 minutes to obtain practical sterility. A few mold spores survived such treatment but caused no serious trouble. Butyric acid bacteria,

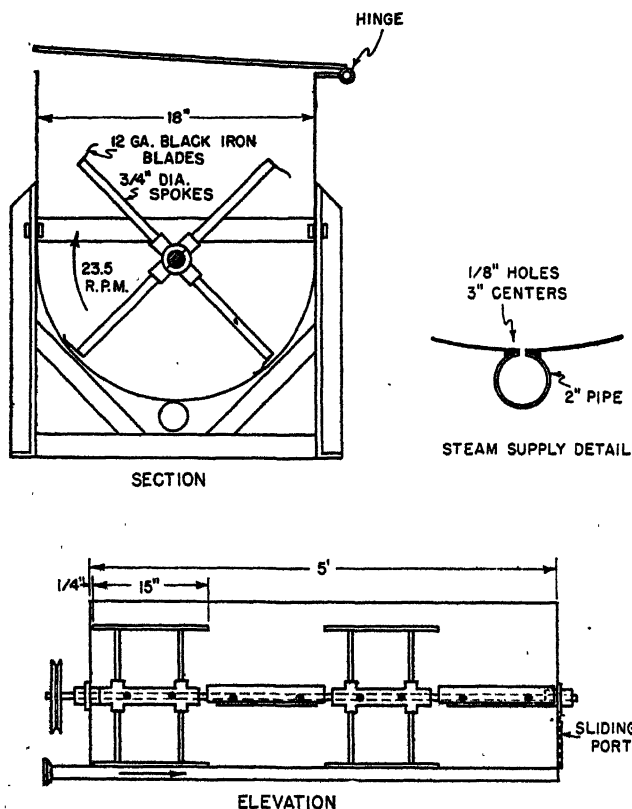


Fig. 1. Cooker detail.

which very badly interfere with mold growth, were eliminated. Hence, a pilot plant cooker was designed employing cooking with steam at atmospheric pressure. This cooker is shown in Figure 1. It consisted of a covered mixer, 18 inches in diameter by 5 feet long. The mixer was nondirectional, had a speed of 23 rpm, and produced efficient agitation in the bran mass. Steam was admitted through a row of jets at the bottom of the cooker. The cooker was of black iron, and no

serious corrosion occurred during operation over a period of several months.

The cooker was operated on a batch system. The bran, in 50-pound lots, was mixed with the acid and steamed in the covered cooker with continuous agitation for 30 minutes. Obviously the amount of dilution due to steam condensation in any installation of this kind would depend upon local conditions of steam quality. Experience with the pilot plant installation showed that use of 0.2 *N* hydrochloric acid, containing double the desired concentration of mineral salts, mixed in the ratio of two parts bran to one part acid resulted in the desired final moisture content of about 51%. Plating tests on the cooked bran showed no growth of foreign microorganisms after incubation for several days. Cooling was accomplished by means of an air stream blowing through the unit, with mixer running, until the temperature dropped to about 35°C. The spore inoculum was then added to the bran in the cooker by means of an insecticide duster, and the cooker was emptied by elevating one end, with mixer running, allowing the expulsion of the inoculated bran from a port in the end of the cooker, the inoculated bran being conveyed in baskets to the incubation units.

*2. Inoculum Development.* The development of an adequate method for producing spore cultures on the laboratory scale has been described above. The laboratory work demonstrated that the amount of bran which can be employed in a container is limited, thin layers being necessary if extensive sporulation is to occur throughout the mass. Moreover, the humidity and air supply must be carefully controlled to achieve most rapid growth, sporulation, and correct rate of drying. A method was still required for producing large amounts of spore inoculum for commercial operation. To accomplish this purpose, galvanized iron pans were constructed, 24 by 35 by 4 inches, provided with covers as shown in Figure 2. The pan cover had an air inlet at the center and air outlets, extending to within an inch of the bottom, at each corner.

For preparation of spore cultures in the pan, 4.5 pounds of the standard corn-bran-acid mixture is spread in it to a depth of about  $\frac{1}{2}$  inch, and the pan with contents is sterilized by heating with steam. The cooled medium is inoculated with mold spores and the pan is placed in a constant temperature cabinet at 32°C; it is desirable to have good air circulation around it. Air at 30° to 32°C is added through the center opening at the rate of 1200 to 1800 ml per minute. At the end of the 4th or 5th day, when heavy sporulation has been obtained, the cover is removed and the pan is placed in the cabinet

again. The spore culture dries within 24 hours and is then ready for use.

3. *Methods for Inoculation.* The method developed for preparing spore inoculum was fully satisfactory for large scale use, but in a large mold bran plant, if 1% inoculum were employed as in the laboratory, the requirement of inoculum per day would necessitate the use of so much spore culture that the inoculum development unit would be of unwieldy size. Two lines of investigation were followed in finding a solution for the inoculation problem. First the value of mycelium

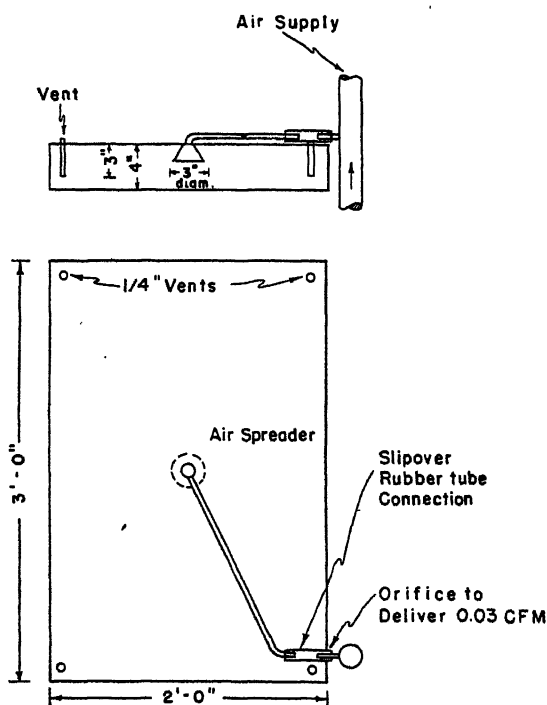


Fig. 2. Inoculum pan.

transfer was studied, and it was found that two mycelium transfers could be used before contamination with other molds, spores of which survived the sterilization, became very serious. However, this scheme for inoculation was inconvenient and a study was next made to determine the influence of the inoculation ratio using spores. No evidence was at hand that the 1% ratio used in the laboratory was either optimum or minimum. To obtain satisfactory spore distribution, an insecticide duster was used to transfer the spores to the bran in a mixer which continually exposed fresh bran to the inoculum, various

ratios of spore inoculation being tried. It was found that even as low an inoculation ratio as 0.04% of dry spore culture was satisfactory, and mold growth was as rapid and as heavy as with higher inoculation ratios. Lower inoculation ratios than 0.04% resulted in slower and less prolific mold growth. It was finally concluded that spore inoculation was definitely superior and more convenient than mycelium inoculation, and, to allow for possible lower spore viability, it was decided to employ 0.10% of dry spore inoculum in all subsequent pilot plant investigations.

4. *Methods of Incubation.* Investigation of methods for incubation of the growing mold and evaluation of the products produced occupied most of the time during the pilot plant research. After trying many testing methods and finding them wanting, the tedious fermentation procedure was used for the final evaluation of the mold brans prepared during the course of this work. To insure comparable results the same sample of wheat was used in all tests. This wheat was one of the lowest alcohol yielders tested in the Nebraska laboratories. Its relative alcohol-yielding capacity in comparison with other grain samples, expressed in terms of grams of ethanol produced per 100 g of total dry matter and employing the regular test method with optimum proportions of malt, was as follows: test wheat, 30.5; official No. 2 hard wheat, 33.5; official No. 2 spring wheat, 32.8; official corn, 36.0.

The details of the fermentation test method were as follows: In each 2-quart square form Mason jar 250 g ground wheat plus 2.5 g malt were mixed with 500 ml water at 63°C, held for 10 minutes at this temperature, and then heated in a water bath at such rate as to raise the temperature to 90°C in 30 minutes. The mash was then heated in the autoclave for one hour at 15 pounds steam pressure. The jars were removed, one at a time, from the autoclave and the mash allowed to cool to 90°C. Then the requisite amount of mold bran (or malt when this was under test) as a slurry in 600 ml of cold water (15°C) was added quickly with rapid stirring. The jar was then placed in a cold water bath, and when the mash temperature reached 32°C it was inoculated with 33 ml of a culture of yeast, No. 567 of the Northern Regional Research Laboratory collection, grown in potato wort. The fermentations were incubated for 72 hours at 32°C, and then analyzed for alcohol. The alcohol yields were based upon loss of weight during incubation and alcohol recovered by distillation.

The laboratory research had shown that when the bran contains the proper moisture and nutrients, and is inoculated at 32° to 35°C with good spore inoculum, the spores germinate in about 3 to 4 hours and the temperature of the bran starts rising in about 5 to 6 hours,



assuming there is sufficient oxygen available for the metabolic processes of the mold. Aeration must be started at that time and it is highly desirable to avoid mechanical disturbance of the bran from that point until the bran particles are well covered, because the young mold mycelium is tender and easily damaged. Oxidative reactions continue at an increased tempo, reaching a peak at about the 15th hour, then declining during the next 15 to 20 hours. Aeration may be employed to maintain proper temperature control; and, to avoid serious drying during this operation, the air should be nearly saturated with moisture.

Incubation of the mold on bran in trays with natural aeration was investigated, using four horizontal trays, as shown in Figure 3, to

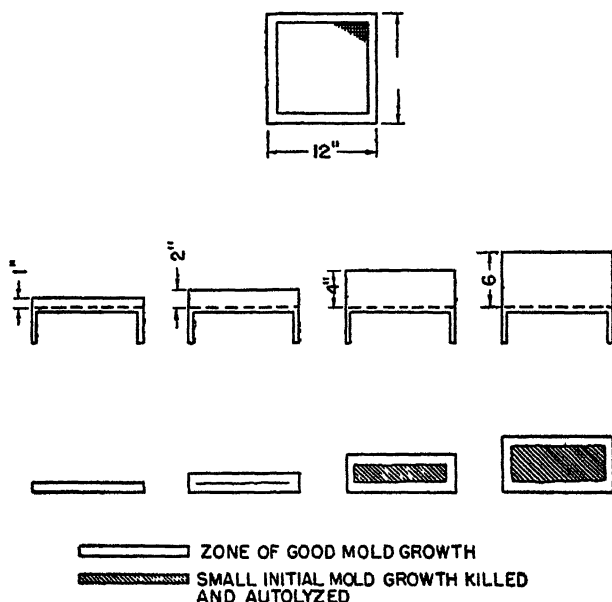


Fig. 3. Tray incubation with natural aeration.

permit study of the influence of layer thickness. These trays were placed in a humidified cabinet after being filled with inoculated bran from the pilot plant cooker, so that natural circulation of air around and through the bran layer was not impeded. The maximum layer depth at which uniformly good mold growth was obtained was 2 inches. With greater bran layer depths, there was a layer of good mold growth about 1 inch thick on the outside, and inside this the temperature reached such high levels that the small initial growth was killed and autolyzed.

The principal virtue of tray incubation is its simplicity. The disadvantage is the tremendous tray area required. In a mold bran unit

for processing one ton of bran per day, provision for 36 hours incubation on the trays would mean a tray requirement for 3000 pounds of bran. Assuming 15 pounds of dry bran per cubic foot (some brans give weights as low as 13 pounds per cubic foot) and a 2-inch bran depth, a tray area of 1200 square feet is required. Assuming trays 6 by 6 feet, there would be required 34 trays, which for this size unit would mean emptying and refilling one tray per hour. This is not unreasonable, but probably a minimum size of commercial plant would expect to process at least 10 tons of bran per day, which would mean about 12,000 square feet of tray area and the handling of about 10 trays per hour if the trays were 6 by 6 feet in size. The mechanical operation of handling so many trays thus becomes unwieldy. Such large tray areas present a handling problem which was not solved during the period of pilot plant work.

The method of incubating the growing mold on thick layers of bran with aeration under pressure was investigated exhaustively. In order to obtain data on the influence of the temperature of incubation and of the moisture content of the bran, it was necessary to develop a small incubation cell by trial and error procedure. After a number of failures, a wooden cell 12 by 12 by 12 inches was built with a false bottom and top, each with a screened air inlet or outlet 4 by 4 inches with a 4-inch unperforated border, and equipped so that air flow could be easily reversed. This cell had a capacity of 10 pounds of dry bran at a 10-inch depth and the false top was designed to fit snugly in the box so that it could be pressed down to rest on top of the bran. Temperatures in various parts of the bran were measured with thermometers inserted through the side of the cell. The air was saturated with moisture at the desired temperature, and pressure was measured by means of open tube manometers. The air pressure was changed by throttling as necessary to maintain a volume of air that would hold the temperature within the desired range and the direction of air flow was reversed at hourly intervals. As the rate of oxidation increased and as the interstices between the bran particles became filled with the mold mycelium, increased air pressures were required to maintain the desired temperatures. A record of the air pressure was kept and it was assumed that since this is a good measure of heat evolution, it is also a good measure of amount and rate of mold growth, up to a point where the amount of mold growth is such as to decrease markedly the permeability of the bran.

It was soon found that temperature of incubation and moisture content of the bran are dependent variables, and a number of runs at several moisture contents had to be made at each temperature of incubation. The influence of the temperature with moisture content

optimal at each level, selected from the numerous runs, is shown in Figure 4. It was concluded that with proper adjustment of moisture, the most rapid mold growth is obtained between 32° and 38°C. At this temperature the best moisture content was secured when 8 parts of 0.1 *N* hydrochloric acid were employed with 10 parts of bran by weight. The moisture content of the bran used was 12%, hence the moisture content of the wet bran as it was placed in the incubator was

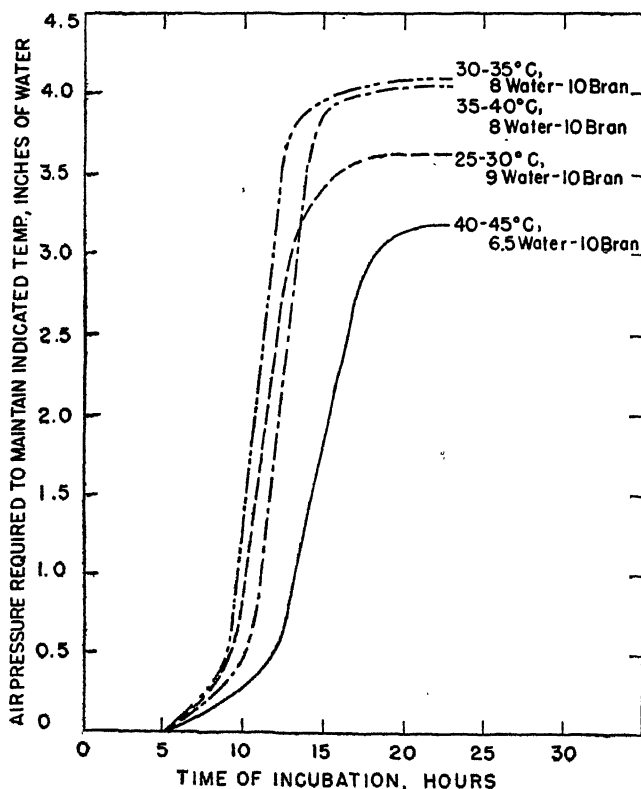


Fig. 4. Influence of temperature upon rate of mold growth.

51%. In all subsequent pilot plant experiments using pressure aeration these conditions of moisture content and temperature were adhered to.

The small horizontal cell gave good mold bran under optimum conditions of temperature and pressure, and duplicate runs gave products which were remarkably uniform as measured by fermentation tests. The effect on the quality of mold bran produced in the horizontal cell when incubated for various times under optimum conditions of

temperature (32° to 38°C) and air pressure to maintain the proper temperature is shown by the data of Table I. It is obvious that, within the limits of experimental error, alcohol yields obtained with the mold bran produced in the small horizontal pilot plant cell on incubation for 36 hours were equivalent to the yields obtained with an excellent laboratory preparation, although somewhat more mold bran was required to obtain the maximum alcohol yield. The data also show that incubation for periods of less than 36 hours resulted in mold bran which had lower saccharifying ability.

TABLE I  
TIME OF MOLD INCUBATION AND ALCOHOL YIELD

Mold bran, %	Ethanol yield, g/100 g total dry matter					Laboratory mold bran
	Mold bran incubated for					
	20 hrs	24 hrs	28 hrs	32 hrs	36 hrs	
0.2	—	—	—	—	—	23.1
0.5	—	—	—	—	—	26.5
0.7	25.5	25.9	27.5	26.0	27.8	—
1.0	26.6	27.3	29.0	28.0	29.5	29.8
1.5	28.1	29.0	29.8	29.0	30.8	30.9
2.0	29.0	29.6	30.3	29.8	31.0	31.5
2.5	29.9	30.5	30.7	30.5	31.1	—
3.0	30.4	30.7	30.9	31.0	31.4	31.6
4.0	30.6	31.2	31.1	31.2	31.4	—

Since the small horizontal cell gave satisfactory mold bran when operated under optimum conditions, it was considered possible that a similar cell of greater size might be employed for larger scale work. However, a larger horizontal cell has several marked disadvantages, the most important being difficulty in emptying.

Another cell arrangement is vertical, which would provide for ease in filling at the top of the cell and for emptying at the bottom. A vertical cylindrical cell proved a complete failure and a vertical cell rectangular in shape was designed with vertical hardware cloth faces through which air under pressure could be forced through the bran in the cell. A modified form of such a cell is shown in Figure 5. Much of the pilot plant work was done with a cell of this type, having a bran cell 3 by 3 by 1 feet in size, with 8-mesh hardware cloth faces and air spaces on each face of the cell. To prevent loss of air around the bran in the cell, an unperforated border was used. This closed the air path for that portion of bran within the borders, but there is a certain amount of diffusion which serves these areas. Obviously there is a limit to the width of these borders. By trial and error method, it was

found that 4 inches was the practical limit of the border, and this width was used in constructing the vertical cell. The first run showed that the bran packed badly at the bottom, and a vertical cell could probably not be more than 3 feet high because of this packing. The best design was found by constricting the bran compartment near the top so that as the bran settled it filled the constricted section and thus reduced the air leakage over the top of the bran, and this modified cell is the one shown in Figure 5.

Later a similar type of incubation cell, also with 9 cubic foot capacity, was constructed, in which the bran cell was inclined at an angle of  $45^\circ$  as shown in Figure 6. This cell was as easy to fill and empty as the vertical cell, and packing was not serious.

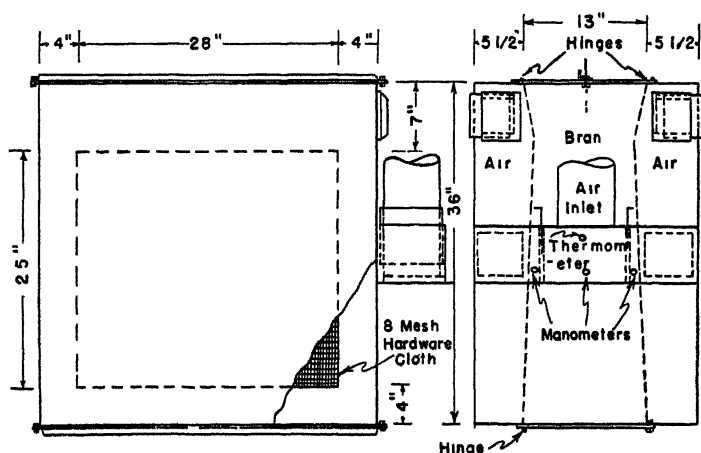


Fig. 5. Vertical incubation cell.

The allowable bran layer thickness is limited by two factors, air pressure required and temperature difference through the layer. Of course the temperature gradient may be changed by changing the air flow, so the two are dependent variables. The maximum temperature difference through a 12-inch layer at the peak of heating, with the rates of aeration described in this paper, was about  $10^\circ\text{C}$ , which is as large as can be tolerated without definite reduction in rate of mold growth. All of the pilot plant cells provided for 12-inch bran layers.

In order to determine accurately the air pressure and volume required, measurements were made using the two types of 9 cubic foot cells described. In Figure 7 are shown graphs of typical data. The incubation temperature was held within the optimum range,  $32^\circ$  to  $38^\circ\text{C}$ , and the moisture content was initially about 51%. It was found that the best control was obtained when the inlet air tempera-

ture was  $30^{\circ}$  to  $32^{\circ}\text{C}$  and the air was 85 to 90% saturated with moisture. The exhaust air temperature reached  $36^{\circ}\text{C}$  at the peak of heating, and at several points in the cell the bran temperature was  $38^{\circ}\text{C}$ .

From graphical analysis of the air volume curve of Figure 7, it may be estimated that the air requirement for 36 hours of incubation averages 32 cubic feet per minute per cubic foot of bran, with a maximum

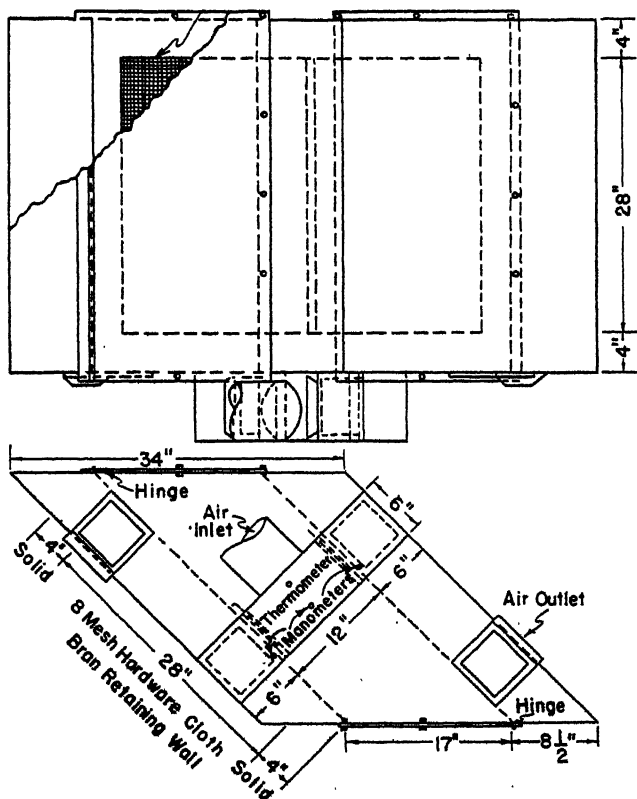


Fig. 6. Inclined incubation cell.

at the peak heating period of 52 cubic feet per minute. These measurements include the air lost by reason of inevitable small leakage in ducts and at the cell.

In the laboratory method for preparing mold bran by incubating in a pan with forced aeration, the bran is removed from the pan shortly after the peak heating period, is broken into small pieces, and held with reduced natural aeration another 24 hours, during which there occurs marked increase in mycelial growth. To investigate the effect of a

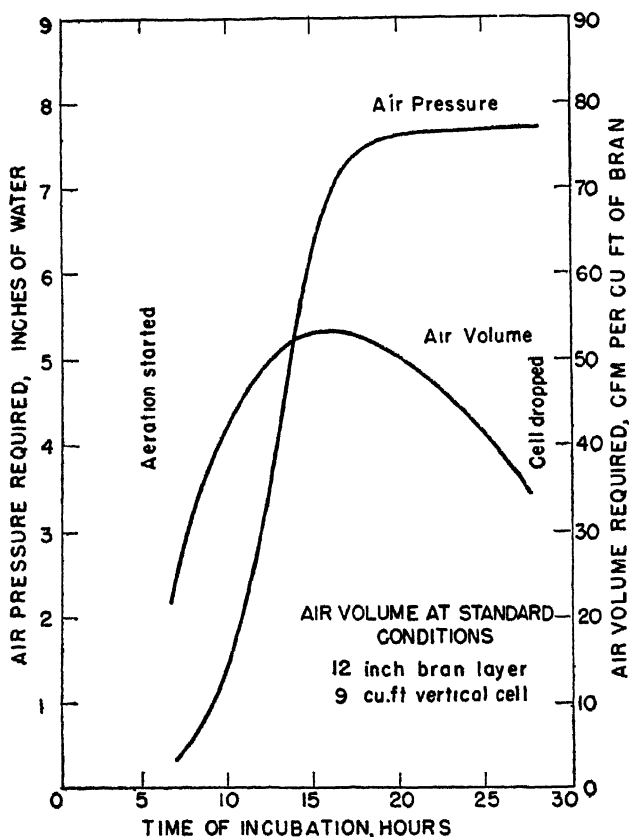


Fig 7. Air pressure and volume requirements during mold growth

similar two-stage incubation system on a larger scale, samples of mold bran were taken from a pilot plant cell after 18 hours incubation, were broken up and then incubated under laboratory conditions to produce very heavy mycelial growth. These were tested by the standard fermentation procedure, yielding the data of Table II. The extra mycelial growth obtained by using the two-stage incubation gave a

TABLE II  
MOLD BRANS WITH HEAVY AND LIGHT MYCELIAL GROWTH—  
36 HOURS TOTAL INCUBATION

Mold bran %	Ethanol yield, g/100 g dry matter	
	Light growth, single stage incubation	Heavy growth, two stage incubation
1.5	27.3	30.0
3.0	30.9	31.9

higher alcohol yield, and less mold bran was required. The data indicate that there is a certain amylase production accompanying the large mycelial production during the secondary incubation stage, although the desired amylases are produced largely during the early stages of growth. The desired amylases are largely extracellular, and in the mold bran must be primarily present in the whole bran mass and not just in the mold mycelium.

Secondary incubation on the pilot plant scale confirmed these results. For pilot plant operations it was necessary to take the mold bran from the primary cell incubator at a point after maximum heat production, which occurs at about the 15th hour, break it into pieces one inch diameter or smaller, and hold it loosely packed in the secondary incubator about 18 to 20 hours. To obtain data on the air pressure and volume requirement for the secondary incubator, a run was made in which the bran was cooked, cooled, and inoculated in the usual manner, and was then incubated in a 9 cubic foot cell for 18 hours at 30° to 35°C. It was then removed from the primary incubator, broken into pieces, and placed in four secondary incubators, consisting of compartments with perforated bottoms, in 1-, 2-, 3-, and 4-foot depths. Air pressure and volume required to maintain the temperature at 26° to 38°C were measured during the 18 hours of secondary incubation.

It was barely possible to maintain temperature control in the 3-foot layer, while the 4-foot layer got completely out of hand. For commercial operation it appeared that a 2- to 3-foot layer should be employed. It was found permissible to use a lower air temperature during this second incubation stage than in the primary, good results being obtained when the incoming humidified air was between 26° and 30°C. The air requirement for the 2-foot depth was 22 cubic feet per minute per cubic foot (12 pounds dry bran) at 6 inches water pressure.

The pilot plant work employing vertical and inclined cells showed that it was feasible to produce mold bran in incubators providing forced aeration through layers of bran, and a design for a commercial plant was developed, employing blocks of inclined cells 6 by 6 by 1 feet in dimensions for primary incubation, with a drag conveyor, or bins with perforated bottoms for secondary incubation.

5. *Usage and Storage.* During the pilot plant work the mold bran was generally dried so that it could be stored and used as needed, because it was convenient to make a number of mold bran products before testing them. It was found that the material containing 25 to 30% moisture as it came from the incubation cells could be dried rapidly because of the large surface exposed, and in the pilot plant the wet product was simply put in bins with false bottoms through which



dry air was blown. The bran layers could be as thick as 4 feet, but it was indicated that the temperature of the air should not exceed about 45°C or there would be a serious loss of amylase. Drying has little effect either upon the amount of mold bran required or upon the alcohol yield obtained. Hence, if mold bran is made in an alcohol plant where it may be used as produced, the recommended practice would be to make up the moist mold bran into a slurry with cold water for use in the plant. This slurry should not be held for more than an hour or two, in the interest of reducing the chance for development of contamination. The concentration of the slurry would, of course, be adjusted to the particular plant requirements. If the mold bran were produced in a separate plant, it would be necessary to dry with a current of warm air, using any suitable type of dryer, to a moisture content below 15%, preferably about 12%. In this dry condition, if protected from outside moisture, the material may be stored in bags or bins for long periods without deterioration.

### **Semicommercial and Commercial Production of Mold Bran**

A semicommercial mold bran unit, with a capacity of about one ton of mold bran per day, patterned after the system developed by the pilot plant research was designed and constructed in the alcohol plant of the Farm Crops Processing Corporation at Omaha. The cooker was similar to that of the pilot plant except that it was 6 feet by 24 inches. Inclined cells were constructed, in a battery, for the primary incubation unit, all with 6 by 6 foot screen dimensions. One of the cells, for experimental purposes, was 8 inches and one was 14 inches between screen faces. Three of the cells were 10 inches, and three were 12 inches between screen faces. As secondary incubators, closed bins with perforated metal bottoms were employed. Separate fans and humidifiers supplied air to the primary and to the secondary incubators.

The primary incubation cells worked fairly well when air control was carefully maintained, but the necessary regulation of air pressure was found to be very critical and temperature control was extremely difficult. Another major difficulty was the shrinkage of the bran, and it was necessary after incubation for a few hours to pack additional inoculated bran into the cells to prevent air leakage over the top of the mold bran "chunks." The secondary incubation was satisfactory. With careful control this semicommercial unit produced mold bran of fair quality, but by no means equal to standard laboratory preparations. The conclusion was reached that it would be unwise to attempt the construction of a larger plant to this design.

As previously mentioned, the simplest incubation system involves the use of trays with a bran depth of not over 2 inches. Shortly after

the semicommercial unit began production, a system for handling the requisite large tray area was conceived, and a new incubator installation was built, incorporating this method. The flow sheet for the operation of this plant, as drawn by Van Lanen and Blom of the Northern Regional Research Laboratory of the U.S.D.A., is shown in Figure 8. In this system the trays were made of standard  $1\frac{1}{2}$  inch iron pipe and approximately 5 by 14 feet in size. The trays were hinged lengthwise so that the front could be dropped for unloading and raised for filling, thus eliminating the labor incident to handling individual trays. The trays were hung in an incubation room built

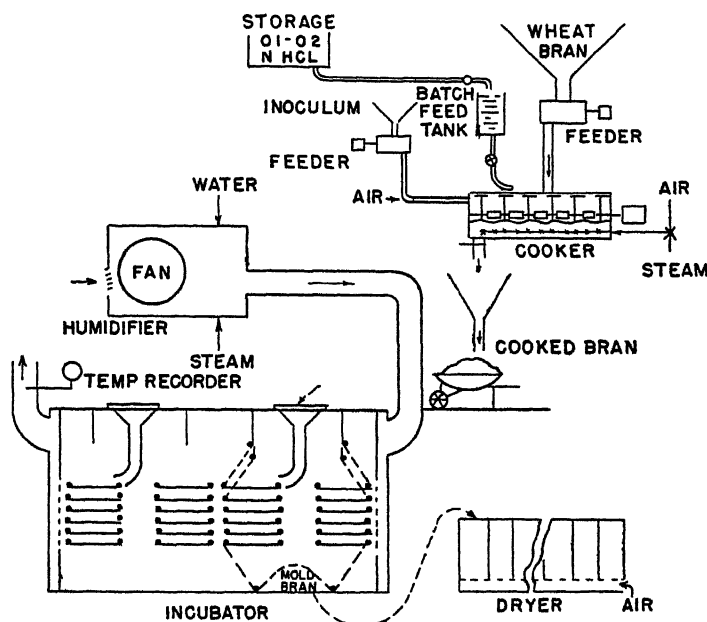


Fig. 8. Flow sheet for semicommercial mold bran production.

of tile and provided with air ducts for circulating humidified air. The same cooker as for the previous installation was employed, and the secondary incubator of the original installation was used, with dry air, for drying the mold bran. Control was simple with this installation and mold growth was excellent, the product being very uniform in quality from day to day, and of high amyolytic activity.

Both the cell unit and the tray unit were operated for a period of about 9 months at the Omaha plant, most of the time simultaneously, in order to produce the quantities of mold bran required for testing its use on the plant scale. These tests were so favorable that one of the government's idle hemp-processing plants, at Eagle Grove, Iowa, was converted to produce mold bran at the rate of 10 tons per day.

Aside from certain mechanical difficulties attendant on the operation of a new plant, the only major difficulty experienced at the Eagle Grove plant has been difficulty in procuring bran. This has resulted in great variability in the bran processed, necessitating frequent changes in the operating conditions as regards moisture, nutrient requirements, and the like. However, commercial operation has demonstrated the soundness and feasibility of the process for the commercial production of mold bran.

### Commercial Uses of Mold Bran

The use contemplated for the mold bran produced at the time of the erection of the Eagle Grove plant was in the manufacture of industrial alcohol from grains. The commercial tests with mold bran in the alcohol plant at Omaha, reported by Underkofler, Severson, and Goering (1946), showed conclusively that alcohol yields were at least as good or somewhat better when mold bran was used instead of or

TABLE III  
RESULTS OF PLANT-SCALE TESTS OF MOLD BRAN

Number of fermenters	Saccharifying agent in fermenter mashes	Saccharifying agent in yeast culture mashes	Average alcohol yield per standard bushel	
			Proof gal	Gal 190 proof
299	10% malt	22% malt	4.77	2.51
847	9 to 10% malt	8.6% malt plus 4.3% mold bran	5.17	2.72
6	4% mold bran	8.6% malt plus 4.3% mold bran	5.24	2.76
12	9 to 10% malt	8.6% malt plus 4.3% mold bran	5.15	2.71
7	3.9-6.2% malt plus 2.2-0.9% mold bran	8.6% malt plus 4.3% mold bran	5.26	2.77
12	9 to 10% malt	8.6% malt plus 4.3% mold bran	5.23	2.75

along with malt for saccharifying the fermentation mashes, and saccharification was accomplished at less cost. Typical plant yield data, taken from the paper of Underkofler, Severson, and Goering (1946), are shown in Table III. Research has indicated also that mold bran may find use in other fermentation processes as a source of enzymes and/or growth factors.

Recently the sugar shortage has resulted in the development of a tremendous demand for enzyme-converted grain syrups, and pilot plant studies were made on the use of mold bran as the converting agent for preparing such syrups. These experiments showed that

better results were obtained when combinations of mold bran and malt were employed than when either was used alone for the saccharification. Such mold bran-malt combinations are now in use for the large-scale commercial production of enzyme-converted grain syrup.

Expanded use of mold bran in other industrial fields using enzymes, such as in production of adhesives, paper sizing, sizing and desizing of textiles, and the like, may be expected, but most probably these industries will employ enzyme concentrates from the mold bran rather than the crude product.

### Summary

Laboratory methods for growing the mold *A. oryzae* on moist, sterilized wheat bran for the production of a low-cost enzyme product, mold bran, have involved growth in a rotating drum, growth on trays in specially humidified and ventilated incubators, and growth in thick layers in a covered pan having a perforated bottom for forced aeration through the bran mass. Thorough sterilization of the moist bran, which can best be effected by using dilute acid and heating with steam, and a period of quiescence during the early growth phases have been found essential for the production of mold bran of highest amylolytic power, and the pan method of incubation was preferred for laboratory work. An efficient method for the production of laboratory spore cultures to serve as inoculum has also been developed.

Pilot plant investigations led to the solution of the main problems in translating laboratory results to commercial production. A cooker was developed for the sterilization, involving a covered mixer with direct steam injection. The sterilized bran was also cooled in the mixer by means of an air stream, and was inoculated in the mixer by blowing dry spore culture into the mixer. It was found that mycelium inoculation was possible, but spore inoculation was preferable, a ratio as small as 0.04% of dry spore culture giving as good results as higher inoculum ratios. Spore cultures for inoculum were best prepared on a large scale by the use of covered pans through which was circulated just enough air to provide for the metabolic requirements of the mold.

Pilot plant investigations of methods of incubation involved growth on trays and in cells with forced aeration. The tray method was simpler, but no satisfactory method of handling the large tray area required for commercial operation was devised during the pilot plant research. A pilot plant method involving the use of either vertical or inclined cells having hardware cloth faces so that air could be forced through the bran was developed. With an initial moisture content of about 51%, most rapid mold growth occurred between 32° and 38°C. The temperature was controlled by varying the rate of air flow through the bran layer by increasing the pressure as required. Two-stage

incubation gave somewhat better mold bran than incubation for the entire period in a single cell.

Semicommercial production of mold bran in two units showed that a unit involving incubation on swinging trays in a specially ventilated room was easier to operate and gave a more uniform product than a unit designed in accordance with the pilot plant method using cells. Optimum conditions of moisture content, nutrient requirements, temperature range, air requirements, incubation time, and the like, ascertained during the pilot plant research with cells hold equally for the process using the swinging trays. A commercial plant processing 10 tons of bran per day is now in successful operation.

Large-scale uses for mold bran have developed in saccharifying grain and potato mashes for the alcoholic fermentation and in saccharifying grain for the production of enzyme-converted syrups.

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# GRAIN STORAGE STUDIES. V. CHEMICAL AND MICROBIOLOGICAL STUDIES ON "SICK" WHEAT<sup>1,2</sup>

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Among the most poorly defined types of damage in wheat associated with storage deterioration is the condition known to the grain trade as "sick" wheat. This damaged condition is manifested by kernels showing a dull appearance with discolored or blackened embryos. Mold growth is usually present on commercial samples of such grain, its viability is low, and the fat acidity is high.

Swanson (1934) found that mold growth on wheat could be inhibited by the exclusion of air from the containers or by treatment of the seeds with Ceresan (ethyl mercury phosphate), and yet sick wheat would develop with time in samples so stored. He also noted that under such conditions, serious damage to baking qualities did take place in the absence of mold growth, but this deleterious effect did not show itself in increased fat acidity.

Thomas (1937), on the other hand, believed sick wheat to be due to toxic principles elaborated by molds, principally the species *Aspergillus flavus*, which grow on wheat and reduce its viability when the moisture content is sufficiently high.

The most comprehensive investigation of this phenomenon is that of Carter and Young (1945) who produced sick wheat in the laboratory by storing Fulcaster wheat in sealed containers at various moisture contents and temperatures over time intervals up to 687 days and followed the changes in fat acidity and the decrease in germination. They arbitrarily eliminated all samples on which molds were visible and concluded that sick wheat formation is associated with anaerobic storage. In general an increase in sick kernels, favored by high moistures and temperatures, was accompanied by an increase in fat acidity and a decrease in germination.

The present study was undertaken to (1) determine the various types of microflora occurring on sick wheat, (2) determine the influence of temperature, moisture, time, and storage atmosphere on the de-

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velopment of sick wheat and associated microfloral contaminants, and (3) follow these changes by indices of chemical deterioration.

### Methods

Quantitative determination of the number of sick or germ-damaged seeds in various samples was made by the Federal Grain Inspection Office, Minneapolis, Minnesota. Assay was made of the number of seeds showing internal microfloral infection, as well as the types present, by a technique involving surface disinfection with sodium hypochlorite and plating the seeds on Smith-Humfeld agar (Smith and Humfeld, 1930). Germination of various wheat samples was determined by the State Seed Testing Laboratory, University Farm, St. Paul, Minnesota, except for special studies for which germination tests on wet filter paper were made.

The technique used for experimental milling of wheat samples, as well as methods of determination of moisture by the two-stage air-oven method, fat acidity, and sugars, is outlined in *Cereal Laboratory Methods* (4th ed., 1941).

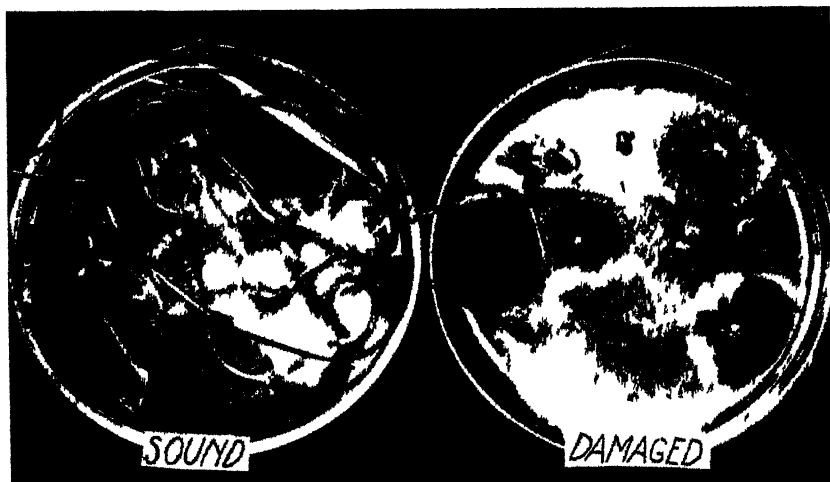


Fig. 1. Sound and germ-damaged wheat from the same lot on Smith-Humfeld agar 10 days after surface disinfection. Most of the sound seeds have germinated, the molds growing from them are *Alternaria*, *Fusarium*, and *Helminthosporium*. None of the germ-damaged seeds have germinated, species of *Aspergillus* and *Penicillium* as well as bacteria are growing from these seeds.

### Germination and Microflora of Sound and Sick Wheat

Small samples of healthy and germ-damaged seed were hand picked by the Federal Grain Inspection Office, Minneapolis, Minnesota, from each of 12 carloads of wheat received from various points in Montana, North Dakota, South Dakota, and Minnesota in February and March,

1945. All of the lots were graded either musty or sour. Fifty seeds of the sound and germ-damaged samples were surface disinfected and placed on Smith-Humfeld agar in petri dishes. Figure 1 is a photograph of typical sound and germ-damaged seed, 10 days after this treatment. Figure 2 is a close-up view of a germ-damaged seed from



Fig 2 Close-up view of a germ damaged wheat seed seven days after surface disinfection (from the same lot as shown in Figure 1, right).

the same lot as shown in Figure 1, right. The percentage of germination and number and kind of molds that grew from each seed are recorded in Table I.

The molds inhabiting the sound seeds, namely, the species of *Alternaria*, *Fusarium*, and *Helminthosporium*, were those commonly present in sound wheat in this region at the time of harvest. None of these are able to grow at a seed moisture content below 25%, and evidence presented elsewhere in this paper indicates that they disap-

TABLE I

PERCENTAGE OF GERMINATION AND INTERNAL MOLD INFECTION OF SOUND AND GERM-DAMAGED WHEAT OBTAINED FROM COMMERCIAL SOURCES <sup>1</sup>

Kind of wheat	Germination	Mold infection	Kinds of molds and percentage of each in the total population
Sound	% 86.7	% 64.5	<i>Alternaria</i> 90%, <i>Fusarium</i> 5%, <i>Helminthosporium</i> 5%
Germ-damaged or "sick"	1.4	76.0	<i>Aspergillus glaucus</i> 60%, <i>Penicillium</i> 20%. Remaining 20% made up of <i>A. niger</i> , <i>A. flavus</i> , <i>A. candidus</i> , <i>Nigrospora</i> , <i>Rhizopus</i> , <i>Trichoderma</i> , and several unidentified bacteria

<sup>1</sup> Average of 12 samples



pear relatively rapidly from seed stored at 18% moisture. (Even at higher moisture contents they appear unable to compete successfully with other molds, notably the *Aspergillus* species, which grew out from the seed. The majority of the molds isolated from the germ-damaged seed, on the other hand, make up the dominant flora which proliferate at moisture contents between 14.5 and 20%, *Aspergillus glaucus* being able to grow on seeds with moisture contents below 16%, *A. candidus* at 17%, *A. flavus*, certain *Penicillia*, and possibly certain bacteria at from 18 to 20%. Some of the minor molds that were present in the germ-damaged seed, such as *Nigrospora*, *Trichoderma*, and *Rhizopus*, do not grow on wheat until a moisture value between 20 to 25% is reached, and their presence in a few of the seeds suggests that the moisture content of at least some individual seeds in these samples had exceeded 20% at some time during storage.

#### Effect on Viability of Inoculation of Sound Wheat with Microorganisms Isolated from Sick Wheat

In studies of the effect of inoculation of sound wheat with microorganisms found in sick wheat on seed viability, at 18% moisture, a sample of irrigation-grown hard red spring Montana wheat (hybrid variety), which was found to be virtually free from internal molds, was used. Portions of the internally mold-free wheat were placed in 8-ounce screw-capped medicine bottles and a 0.5% aqueous solution of sodium hypochlorite was added to eliminate microorganisms from the outside of the seed as well as to bring the moisture content up to 15%. After 24 hours, sterile water in which mold spores or bacteria were suspended was added to bring the moisture content up to 18%. Three molds, namely *A. candidus*, *A. glaucus*, and *A. ochraceus*, and four unidentified bacteria isolated from sick wheat were tested. Three controls were used, all of which received the preliminary surface disinfection described above when the moisture content was raised to 15%. To one, only sterile water was added to raise the moisture content from 15 to 18%; the moisture content of the second was raised from 15 to 18% with a 1% solution of sodium hypochlorite; the third was identically treated as the first, but after the water was absorbed it was dusted with 0.2 g chloramine B per 100 g of seed. Three replicate bottles of each control and of each inoculated sample were used. The bottles were stored at room temperature (22° to 26°C) and samples were removed after 38, 111, and 201 days, surface disinfected, and placed on sterile moist filter paper to determine the germination. The results are summarized in Table II. It should be emphasized that the methods of surface disinfection used did not eliminate all the microorganisms in the seed of the controls.

TABLE II

VIABILITY OF MONTANA WHEAT INOCULATED WITH VARIOUS ORGANISMS AND STORED AT A MOISTURE CONTENT OF 18% FOR VARIOUS LENGTHS OF TIME

Treatment	Percentage of germination after		
	38 days	111 days	201 days
Control, 0.5% sodium hypochlorite	93	49	0
Control, 1.0% sodium hypochlorite	91	67	0
Control, 0.5% sodium hypochlorite plus dusting with Chloramine B (0.2 g/100 g)	90	67	0
Bacterium No. 1	92	42	0
Bacterium No. 2	95	29	0
Bacterium No. 3	84	36	0
Bacterium No. 4	91	19	0
<i>Aspergillus candidus</i>	36	14	0
<i>Aspergillus glaucus</i>	74	35	0
<i>Aspergillus ochraceus</i>	34	24	0

The three molds were tested again on the Montana wheat, but this time at 20% moisture after storage for 22 days, germination being determined on agar and in nonsterile soil. The viability of the inoculated and noninoculated seeds is given in Table III.

In both tests *A. candidus* and *A. ochraceus* reduced the viability of seeds rather rapidly, while *A. glaucus* reduced the viability somewhat more slowly. These molds produce heavy tufts of sporophores on the germ ends of the seeds in six to eight days at moisture contents of 16 to 20%, although later sporophores may arise from the entire surface of the seeds. Typical seeds stored at 16% moisture for 10 days are illustrated in Figure 3. In both tests, most of the dead seeds had the dark germ which is typical of sick wheat.

The role of bacterial growth in the loss of viability of these stored samples is less clear than that of the molds. The multiplication of bacteria on and in the seed is less easy to follow by macroscopic and microscopic examination than is the case with the molds. In fact,

TABLE III

VIABILITY OF MONTANA WHEAT INOCULATED WITH THREE DIFFERENT MOLDS AND STORED FOR 22 DAYS AT 20% MOISTURE

Treatment	Percentage germination after 22 days	
	On agar	In nonsterile soil
Control, surface disinfected	100	82
Inoculated with:		
<i>Aspergillus candidus</i>	48	29
<i>Aspergillus glaucus</i>	76	71
<i>Aspergillus ochraceus</i>	54	52

no external evidence of bacterial proliferation was evident on seeds stored at these moisture values. The results of Table II do, however, suggest that Bacterium number 4 and possibly numbers 2 and 3 contributed to the deterioration of wheat stored at 18% moisture, and tests now under way indicate that these bacteria seem to multiply slowly on or in autoclaved and subsequently inoculated wheat held at a moisture content of 18%. Further evidence on this point will appear in a later portion of this paper

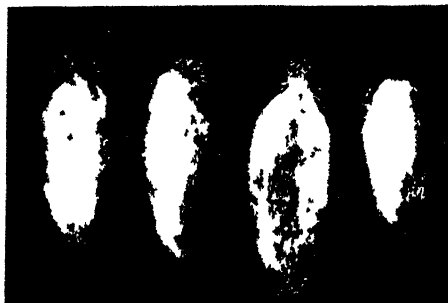


Fig 3 Sound, surface disinfected wheat seeds stored for 10 days at 16% moisture. Tufts of *Aspergillus glaucus* sporophores are growing out of them, chiefly from the germ end

### **Influence of Moisture, Temperature, Time, and Interseed Atmosphere on the Development of Sick Wheat and Contaminating Microflora**

To secure data on the influence of various environmental factors on the development of sick wheat, an experiment was carried out in which wheat was stored at two moisture and temperature levels under different gaseous atmospheres. For this study a lot of sound Regent seed wheat, grown at McIntosh, South Dakota, in 1944, showing 94% germination was used. Two lots of wheat were conditioned to 12.1 and 18.0% moisture, and 500-g portions of each lot were distributed among a number of ordinary quart-size mason jars. The regular flat metal covers were perforated and one-inch lengths of  $\frac{1}{16}$ -inch copper tubing were soldered to these openings. After sealing with the metal screw ring, closure was effected by means of a short length of neoprene tubing and a screw clamp. Each lot of every moisture level was divided into three groups of equal number. Atmospheres of carbon dioxide, nitrogen, and oxygen were then introduced by evacuating the air in the jars with a vacuum pump followed by introduction of the commercial compressed gases. This procedure was repeated for each jar to ensure virtually complete displacement of the air by the gas. The various jars were then stored for six months at two temperatures: room temperature (22° to 26°C) and 5°C. The grain stored at room

temperature was sampled and assayed for germination, sick kernels, fat acidity, and sugars at monthly intervals. Samples at 5°C were examined at three-month intervals. Extensive microfloral examination was carried out on the samples stored at room temperature at the end of two and five months.

After two months' storage, samples of the gaseous atmosphere in the jars containing nitrogen and oxygen, which were stored at room temperature, were withdrawn and analyzed for carbon dioxide using the Haldane-Henderson gas analyzer. A high concentration of this gas was found and this necessitated the refilling of the balance of the oxygen and nitrogen jars of this moisture group at monthly intervals with fresh gas. The concentration of carbon dioxide after monthly periods is shown in Table IV.

TABLE IV  
CONCENTRATION OF CARBON DIOXIDE APPEARING IN JARS CONTAINING REGENT  
WHEAT STORED AT 12.1 AND 18.0% MOISTURE UNDER  
INITIAL ATMOSPHERES OF NITROGEN AND OXYGEN

Time of sampling	Storage temperature	Carbon dioxide in interseed atmosphere			
		Moisture 12.1%		Moisture 18.0%	
		Nitrogen atmosphere	Oxygen atmosphere	Nitrogen atmosphere	Oxygen atmosphere
<i>Months</i>		%	%	%	%
2	Room	0.8	0.9	35.1	92.4
3	Room	0.5	13.9	21.2	97.6
4	Room	0.6	0.4	8.3	79.9
5	Room	0.5	0.7	5.2	88.1
3	5°C	0.1	0.1	5.2	4.9

Rather rapid respiratory exchange of oxygen to produce carbon dioxide can apparently occur in sealed containers in one month of storage at 18% moisture. Even under anaerobic conditions (nitrogen atmosphere) considerable carbon dioxide production occurs. Had the jars contained only normal air (20.9% oxygen), oxygen depletion would be expected to occur even more rapidly.

Data for fat acidity, reducing, nonreducing, and total sugars, germination, and percentage of sick or germ-damaged kernels in the various samples are given in Table V. Results of the microbiological examination at the end of the second and fifth months of storage obtained by surface-disinfecting 50 seeds of each lot with sodium hypochlorite, plating on sterile Smith-Humfeld agar, and incubating at room temperature for 10 days are shown in Table VI.

Chemical deterioration, both as regards lipolysis and sugar hydrolysis, was more rapid in the samples at 18% moisture than at 12.1%

TABLE V

INFLUENCE OF MOISTURE CONTENT, TEMPERATURE, TIME, AND INTERSEED  
ATMOSPHERE ON THE CHANGES IN CHEMICAL COMPOSITION,  
GERMINATION, AND FORMATION OF SICK KERNELS  
IN REGENT WHEAT

Time of sampling	Storage temperature	Initial moisture 12 1%			Initial moisture 18 0%		
		CO <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>
Months      FAT ACIDITY, MG KOH PER 100 G DRY WHEAT							
0	Room	13.8	13.8	13.8	13.8	13.8	13.8
1	Room	17.8	19.7	23.0	18.8	20.2	25.7
2	Room	24.1	24.1	22.6	44.3	22.7	31.3
3	Room	36.2	35.3	35.2	40.3	39.1	54.1
4	Room	24.1	23.2	23.3	26.3	25.2	46.3
5	Room	19.2	19.3	19.3	23.4	22.7	34.7
6	Room	23.2	23.3	23.3	29.4	31.6	40.9
3	5°C	35.3	30.4	32.8	39.6	44.6	43.3
6	5°C	21.7	21.7	21.1	21.1	20.5	24.2
REDUCING SUGARS AS MALTOSE MG PER 10 G							
0	Room	51	51	51	51	51	51
1	Room	46	51	44	56	54	54
2	Room	40	44	45	60	66	58
3	Room	40	31	32	57	58	55
4	Room	49	49	49	109	101	87
5	Room	46	48	49	119	119	94
6	Room	44	44	43	116	126	93
3	5°C	28	31	32	45	39	35
6	5°C	41	41	43	48	50	49
NONREDUCING SUGARS AS SUCROSE MG PER 10 G							
0	Room	221	221	221	221	221	221
1	Room	207	214	214	202	193	187
2	Room	210	216	213	169	169	167
3	Room	176	143	143	107	117	101
4	Room	230	226	218	138	128	119
5	Room	222	227	242	124	118	119
6	Room	218	208	220	114	126	106
3	5°C	127	143	135	170	150	129
6	5°C	214	217	216	215	196	199
TOTAL SUGARS, MG PER 10 G							
0	Room	272	272	272	272	272	272
1	Room	253	265	258	258	247	241
2	Room	250	260	258	229	235	225
3	Room	216	174	175	164	175	156
4	Room	279	275	267	247	229	206
5	Room	268	275	291	243	237	213
6	Room	262	252	263	230	252	199
3	5°C	155	174	167	215	189	164
6	5°C	255	258	259	263	246	248

TABLE V—*Continued*

Time of sampling	Storage temperature	Initial moisture 12.1°C			Initial moisture 18.0°C		
		CO <sub>2</sub>	N	O	CO	N	O
Month							
REDUCING SUGARS AS % TOTAL, %							
0	Room	18.8	18.8	18.8	18.8	18.8	18.8
1	Room	18.2	19.2	17.1	21.7	21.9	22.4
2	Room	16.0	16.9	17.4	26.2	28.1	25.8
3	Room	18.5	17.8	18.3	34.8	33.1	35.3
4	Room	17.6	17.8	18.4	44.1	44.1	42.2
5	Room	17.2	17.5	16.8	49.0	50.0	44.1
6	Room	16.8	17.5	16.3	50.4	50.0	46.7
3	5°C	18.1	17.8	19.2	20.9	20.6	21.3
6	5°C	16.1	15.9	16.6	18.3	20.3	19.8
GERMINATION, %							
0	Room	94	94	94	94	94	94
1	Room	94	96	94	92	95	86
2	Room	97	98	97	76	69	63
3	Room	93	94	91	5	20	17
4	Room	93	95	91	0	0	0
5	Room	92	90	94	0	0	0
6	Room	96	94	90	0	0	0
3	5°C	90	91	91	93	92	94
6	5°C	97	97	97	93	92	90
SICK WHEAT, %							
0	Room	0	0	0	0	0	0
1	Room	0	0	1	4	2	7
2	Room	0	0	0	1.5	2	6.5
3	Room	0	0	0	25	30	70
4	Room	0	0	0	100	100	100
3	5°C	0	0	0	3	3	1.5

moisture. In addition, it is significant that the wheat stored under oxygen at the high moisture value showed considerably higher fat acidity values as the experiment progressed than did the samples at the same moisture value stored under carbon dioxide or nitrogen. Nevertheless, the development of sick kernels proceeded under the three atmospheres, all being 100% sick at the end of four months. Data for the third month, however, suggest that samples under oxygen tend to become sick somewhat more rapidly than in the other atmospheres. Total sugars showed a somewhat greater decrease in the 18% moisture sample stored under oxygen than was found in the other gases. The reason for these differences is indicated in Table VI; only under the oxygen atmosphere at 18% moisture did significant increases in

*Aspergillus glaucus* and *A. candidus* occur. These fungi are vigorous respiratory and lipolytic agents and, being strictly aerobic in their respiratory requirements, proliferate only in an atmosphere containing oxygen. It therefore seems that the biochemical activity of molds is additive, or synergistic with, other agencies which may be responsible for the formation of sick wheat. Thus, under natural conditions of storage, their deteriorative action would enhance the rate at which sick wheat develops. These fungi occurred on all samples of naturally formed sick wheat.

TABLE VI

EFFECT OF TIME ON INTERNAL MICROFLORAL POPULATION OF REGENT WHEAT STORED AT 12.1 AND 18.0% MOISTURE UNDER ATMOSPHERES OF CARBON DIOXIDE, NITROGEN, AND OXYGEN AT ROOM TEMPERATURE

Time of sampling	Moisture at sampling	Atmosphere	Percentage of seeds internally infected						
			Total	Bacteria	<i>A. glaucus</i>	<i>A. candidus</i>	<i>A. flavus</i>	<i>Alternaria</i>	Other fungi
months	%								
2	12.1	CO <sub>2</sub>	32	—	—	—	—	30	2 (unknown)
	11.9	N <sub>2</sub>	32	—	—	—	—	32	—
	11.6	O <sub>2</sub>	30	—	—	—	—	30	—
	17.8	CO <sub>2</sub>	0	—	—	—	—	—	—
	18.0	N <sub>2</sub>	4	—	—	—	—	2	2 (unknown)
	18.0	O <sub>2</sub>	2	—	—	—	—	2	—
5	12.6	CO <sub>2</sub>	10	—	—	—	—	10	—
	12.2	N <sub>2</sub>	24	—	—	—	—	24	—
	12.4	O <sub>2</sub>	18	—	—	—	—	18	6 ( <i>Penicillia</i> )
	18.4	CO <sub>2</sub>	82	82	2	—	—	—	—
	18.4	N <sub>2</sub>	68	68	—	8	—	—	—
	18.5	O <sub>2</sub>	100	90	50	24	2	—	—

The fact that a large proportion of the sick seeds showed bacterial contamination at the end of five months which was not apparent after two months of storage suggests that either the proliferation and biochemical activity of the microflora may be responsible for the sick condition, or that the progressive loss of viability and increasing chemical deterioration of the seeds due to other factors make it possible for the bacterial inoculum initially present to grow more readily. The literature on the minimal moisture requirements of microflora, which has been reviewed by Milner and Geddes (1946), would suggest that bacterial proliferation is not to be expected at relative humidities below 95%. The equilibrium relative humidity for wheat at 18% moisture is approximately 86%, according to the data of Coleman and Fellows (1925) and Rozsa (1935).

As previously mentioned, the common seed contaminant *Alternaria* rapidly disappears in the presence of moisture conditions favorable to the proliferation of other species such as the *Aspergilli*.

### Distribution of Fat Acidity in the Milled Fractions of Sound and Sick Wheat

After six months, the remaining wheat samples stored at room temperature were experimentally milled. As a control, a sample of the original dry Regent wheat which had been stored at approximately 5°C in contact with a normal air atmosphere was milled. The various milling fractions were then analyzed for fat acidity with the results shown in Table VII.

The difference between the distribution of fat acidity in the various fractions of the normal or nonsick wheats in comparison with those from the sick wheats is very striking. The control sample, and those stored at 12.1% moisture under carbon dioxide, nitrogen, or oxygen, in which no sick wheat developed, shows the highest concentration of fat acidity in the bran fraction; the acidity decreases regularly in the order of mill fractions removed by successive stages of refinement, i.e., bran, brown shorts, white shorts, low grade, clear, and patent. In contrast, the fat acidity distribution in the milled fractions of sick wheat increases from the bran (which, except for the sample stored under oxygen and on which mold growth occurred, had a value comparable to the normal wheat brans) to a high maximum peak in the low grade flour fraction, followed by a regular decrease in the clear and patent flour. It is to be noted that while the milling yield of similar fractions from the various samples differed somewhat, the variation was not of a magnitude such as to affect the identity of the fraction. Hence the corresponding fractions may be considered to have approximately the same fat content, and thus the fat acidities can be logically compared.

While the total fat content of the various fractions was not determined, due to an insufficient sample, the considerable information in the literature on the distribution of total fat among commercial mill fractions of comparable wheats (Sullivan *et al.*, 1927, 1928, 1940, and Barton-Wright, 1938) shows that total ethyl or petroleum ether extract falls regularly with increasing refinement, and that the fat content of the low-grade flour fraction is usually only little more than one-half of that of bran. The fat acidity values in the normal or nonsick wheats analyzed appear to parallel these expected total fat contents rather closely.

### Discussion

These studies are primarily of an exploratory nature but they indicate that a number of complex variables are probably involved in the formation of sick wheat. They indicate that the deterioration is not solely a result of the proliferation and metabolic activity of molds such as *Aspergillus glaucus*, *A. flavus*, and *A. candidus* which are



TABLE VII

FAT ACIDITY OF MILLED FRACTIONS OF REGENT WHEAT STORED FOR SIX MONTHS  
AT ROOM TEMPERATURE AT 12.1 AND 18.0% MOISTURE UNDER ATMOSPHERES  
OF CARBON DIOXIDE, NITROGEN, AND OXYGEN

Storage moisture	Storage atmosphere	Sick kernels	Fraction	Milling yield of fraction	Fat acidity <sup>1</sup> (an-dry basis)
%		%		%	
Control	Air (5°C)	0	Bran	8.7	69.4
			Brown shorts	5.9	61.2
			White shorts	6.4	41.1
			Low grade	0.8	42.9
			Clear flour	1.3	33.3
			Patent	76.9	20.1
12.1	Carbon dioxide	0	Bran	10.1	74.9
			Brown shorts	4.8	69.9
			White shorts	6.1	49.3
			Low grade	0.9	46.6
			Clear flour	1.9	33.3
			Patent	76.2	22.4
12.1	Nitrogen	0	Bran	9.8	70.3
			Brown shorts	6.3	64.4
			White shorts	5.8	45.7
			Low grade	0.9	44.3
			Clear	2.0	32.4
			Patent	75.2	21.0
12.1	Oxygen	0	Bran	11.4	63.9
			Brown shorts	4.7	55.7
			White shorts	5.5	45.7
			Low grade	0.9	42.9
			Clear	1.8	32.4
			Patent	75.7	21.5
18.0	Carbon dioxide	100	Bran	9.7	73.1
			Brown shorts	5.1	79.0
			White shorts	4.6	89.6
			Low grade	1.0	92.7
			Clear	1.8	41.6
			Patent	77.8	24.8
18.0	Nitrogen	100	Bran	11.3	73.1
			Brown shorts	4.1	77.2
			White shorts	4.7	95.9
			Low grade	1.0	127.0
			Clear	1.3	49.3
			Patent	77.6	21.0
18.0	Oxygen	100	Bran	8.3	93.1
			Brown shorts	6.3	115.6
			White shorts	4.8	199.1
			Low grade	0.8	257.1
			Clear	1.8	188.6
			Patent	78.0	39.2

<sup>1</sup> Mg KOH per 100 g.

indigenous to normal wheat, since wheat stored at high moisture under carbon dioxide and nitrogen where mold growth was inhibited became sick to the same extent as did that under an oxygen atmosphere where these molds proliferated. The deteriorative effect of mold growth is manifested principally in lipolytic activity and appears to be additive to other deteriorative processes responsible for sick wheat. In this regard, the conclusion of Carter and Young (1945) that the development of sick wheat may occur in an anaerobic storage atmosphere seems to receive confirmation. Certainly the inference of Thomas (1937) that sick wheat is primarily due to the metabolic activity of molds is not supported by the evidence in the present studies. However, the deteriorative effect of molds usually present under natural conditions of storage enhances sick wheat formation.

The fact that the respiratory activity of wheat stored in sealed containers at room temperature caused almost quantitative exchange of oxygen for carbon dioxide over relatively short intervals indicates that atmospheres must be controlled if experimental results are to be related to natural or commercial storage conditions. Milner and Geddes (1945) have shown that in a commercial bin, zones of heating in stored soybeans, which are centers of high respiratory activity, contain air which is nearly normal in oxygen content owing probably to convection effects at the heating centers. [The gaseous composition of the atmosphere surrounding stored seeds is therefore of critical importance in determining the course of their deterioration.

(The data presented in Table V suggest that bacteria are involved in the sick wheat phenomenon, as the viability of seeds treated with them is reduced more than control samples at the same moisture value. However, the moisture value at which this occurred (18%) is considerably greater than the lowest moisture level (12.2%) at which Carter and Young (1945) noted sick wheat formation after 279 days of storage at 40°C. It seems very unlikely that bacteria could be involved in the deterioration at such a low moisture value. As previously indicated, several workers who have classified microflora on the basis of minimal humidity requirements for growth agree that bacterial proliferation is not to be expected at relative humidity values below 95%. Nevertheless, the present data obtained with bacterial inoculation suggest that attention will need to be given to the possibility of bacterial growth at humidity levels lower than the data of these workers have shown.

(The probability that the basic cause of the sick wheat condition, manifested by darkening of the embryo and eventually of the whole kernel, may be due to the inherent seed enzymes cannot be dismissed. Such enzymic activity may lead initially to a weakening of the germina-

tive powers and eventually to sufficiently extensive chemical deterioration to favor the proliferation of bacteria initially present only as a contaminating inoculum. That the growth of microflora on seeds is enhanced by germinative and chemical deterioration of seeds is apparent from the data of Milner and Geddes (1945a) who showed that soybean seed damaged by storage at a temperature (50°C) which allowed survival of mold spores yielded respiratory values at 30°C greatly in excess of those given by the same seed and contaminating molds stored at 30°C prior to the heat treatment.

The data of Johnson and Hagborg (1944) are pertinent in connection with the darkening which develops on sick wheat kernels. They found that at high temperature, especially when combined with a high humidity, melanistic areas may develop on the glumes, lemmas, peduncles, and internodes of Apex and Renown wheat in the absence of any infection by pathogenic organisms. The data of Johnson and Hagborg suggest that enzymic or even such nonbiological effects as interaction between carbohydrates and nitrogen-containing compounds (Maillard or "browning" phenomenon) are possible under such conditions.

That the darkening of sick wheat, which appears first in the embryo portion where high enzymic activity is to be expected, is probably not related to the increase in fat acidity is indicated by the data of Table VII. The fact that of the various mill fractions of sick wheat, the low-grade flour showed the highest fat acidity value, whereas the bran of normal wheats was highest in this factor in comparison to all other fractions, suggests that the lipolytic agencies in sick wheat do not occur primarily in the bran or in the germ (which accompanies the bran and brown shorts fractions in milling, and which has the highest fat content of any of the wheat tissues), but more probably in the aleurone and scutellar tissues. These tissues show a more organized active cellular structure than do other wheat tissues, and are credited with enzymic functions which become highly active on germination of the seed. The present results are directly in accord with the work of Sullivan and Howe (1933) who investigated the lipase activity of various milling fractions of normal wheat and found it to be concentrated in greatest amount in the clear and low-grade flour fractions. That fungi beneath the bran coat are probably not responsible for the lipolytic activity in sick wheat (except in the presence of oxygen as shown by this study) is suggested by the work of Oxley and Jones (1944) who found the mold contamination to be localized just inside the outermost bran tissue.

Further studies on the phenomenon of sick wheat will need to take into account the nature and distribution in the wheat tissues of various enzyme systems such as the lipases, esterases, and oxidizing enzymes

such as tyrosinase, as well as the moisture relationships which govern their activity. The exact role of bacteria in this phenomenon needs considerable clarification, and it would be of great advantage to grow wheat entirely free from contamination by molds and bacteria for such studies.

### Summary

"Sick" or germ-damaged wheat samples showed very low germination values and were found to be infected principally with *Aspergillus glaucus*, *Penicillium* spp., and to a lesser extent by *A. candidus*, *A. flavus*, *A. niger*, and a few bacteria. In contrast, sound wheat samples from the same lots of grain were largely contaminated with *Alternaria* which disappeared when stored under moisture conditions favorable to the proliferation of the *Aspergilli*.

Surface-disinfected, relatively mold-free Montana wheat inoculated with various molds and bacteria isolated from sick wheat and stored in air lost viability faster than did noninoculated controls, and most of the nonviable seeds had symptoms typical of sick wheat. Molds were present on the noninoculated controls before the test was completed. The influence of bacteria was not so clearly defined as was the effect of the molds.

Sick wheat was produced in the laboratory by storing Regent wheat at 18% moisture under atmospheres of carbon dioxide, nitrogen, or oxygen, in sealed containers. Only under oxygen did molds, principally *Aspergillus glaucus* and *A. candidus*, proliferate throughout the period of the test, whereas sick kernels appeared under all atmospheres. [Fat acidity increased in all samples at 18% moisture, but was greatest in the samples stored under oxygen.] The sick wheat condition, therefore, was not entirely due to molds but their metabolic activity enhanced sick wheat formation.

Aerobic and anaerobic production of carbon dioxide by molds and seeds at 18% moisture in the sealed containers required renewal of the oxygen and nitrogen atmospheres at monthly intervals.

[Fat acidity of mill fractions of sound wheat was at a maximum in the bran fraction, and decreased regularly toward the patent flour, more or less in the order of the total fat content of the various fractions. The milled fractions of sick wheat, on the other hand, showed highest fat acidity in the low-grade flour. The high lipase activity of the aleurone layer and scutellum, relative to that of the germ, is apparently responsible for this effect.

More information is needed on the location, distribution, and activity of inherent wheat enzymes, such as the lipases and oxidizing enzymes, to explain the formation of fat acidity and the darkening of sick wheat.

### Acknowledgment

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# REPORT OF THE 1945-46 COMMITTEE OF THE NEW YORK SECTION ON PROCEDURES FOR THE EXAMINATION OF FLOUR FOR EXTRANEEOUS MATERIALS

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The food industry is continually confronted with new problems in sanitation. As more information is obtained, the problems are recognized and, in general, conscientious efforts are made to attain improvements. Sanitary precautions in handling and preparing food products pay dividends by the improvement of quality, elimination of waste, and protection of the consumer. To insure a high standard of quality, the analyst is called upon to develop practical methods and techniques for the detection of sources of contamination, which may be present but are not detectable by macroscopic examinations.

The executive committee of the New York section of the American Association of Cereal Chemists realized the need to familiarize the industrial analyst with procedures for the examination of food products for extraneous materials. A committee of chemists was appointed in November, 1945, to make a study of procedures available, and, if possible, to recommend those most adaptable to the needs of the average industrial analyst. The importance of simplicity and rapidity in the procedures was recognized, since in most industrial laboratories engaged in ingredient control, the analyst must be prepared to handle a large number of samples daily.

In order to make the procedures practical, it was deemed necessary to condense instructions and simplify manipulations as much as possible and still have a reasonable assurance that, when followed by the analyst, a separation of most of the extraneous materials would be obtained.

As the committee was composed of cereal chemists, procedures for the examination of flour were given paramount consideration. Various procedures were suggested and collaborative examinations of samples were made. The clearest fields which contained the greatest amount of extraneous materials were obtained by making gasoline and mineral oil flotation separations from charges digested with pancreatin and hydrochloric acid.

The training of the analyst and his ability to identify extraneous materials must always be taken into consideration; but with the aid of instructions which have been published recently by the Food and Drug

Administration<sup>1</sup> and the Association of Official Agricultural Chemists,<sup>2</sup> the average industrial analyst should be able to follow these suggested procedures for the examination of flour and obtain a reasonably accurate evaluation of the amount and kind of extraneous materials which it contains.

Two methods of digestion of the flour were studied: one by pancreatin, the other with hydrochloric acid. Gasoline and light mineral oil were the two flotation media used. The pancreatin digestion gasoline flotation procedure is especially applicable to whole wheat and graham flours. The mineral oil flotation tends to trap considerable bran and thereby produce fields which are difficult to examine. With white flours, the hydrochloric acid digestion and mineral oil flotation procedure is rapid and simple in manipulation. Pancreatin digestion and gasoline flotation can also be applied to white flour if preferred, and if the over-all time required to obtain results is not too important.

Description of essential apparatus, a list of required reagents, pertinent information, and the procedures follow:

### Examination of Flours for Extraneous Materials

#### APPARATUS

- (a) Sieve—10 mesh U. S. Standard.
- (b) Special separatory funnel (Figure 1)—1 liter capacity Squibb pear-shaped without ground-in glass stopcock. The lower end should shape down evenly and seal onto a glass stem approximately 3" long with a bore of approximately  $\frac{5}{16}$ ". A piece of rubber tubing is fitted over the stem and a pinch clamp used for releasing and closing the funnel. A rubber stopper is most satisfactory for stoppering the top of the funnel.
- (c) Wildman trap flask (Figure 2)—1-2 liter Erlenmeyer flask into which is inserted a close-fitting rubber stopper supported on a stiff brass rod of approximately  $\frac{3}{16}$ " diameter and 2-3" longer than the height of the flask. Rod is threaded at lower end and fitted with nuts and washers to hold the stopper tightly in place. Lower nut must be countersunk into the rubber to prevent striking the flask. All metal fittings should be brass or a metal which does not corrode readily in salt solutions or in slightly acid or alkaline solutions.
- (d) Filtration funnels and accessories—Büchner or Hirsch funnels for filtration with suction. Funnel should be of such diameter that filter papers will fit closely against the sides. A close

<sup>1</sup> Microanalysis of Food and Drug Products. Food and Drug Circular No 1, 1944.

<sup>2</sup> Official and Tentative Methods of Analysis, Chapter 42, 6th ed., 1945.

fitting disc of bolting cloth placed between the perforated funnel plate and filter paper accelerates filtration and when properly fitted helps prevent possible loss of material around the edges of the paper. Filter papers should be rapid acting. They should be ruled with parallel lines at such a distance apart that the field from line to line is visible under the microscope at the magnification at which the examination is being made. Paper ruled with parallel lines 7 mm apart (such as 7 mm ruled Shark Skin supplied by Schleicher & Schüll Co.,

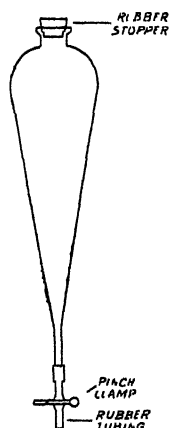


Fig. 1. Special separatory funnel.

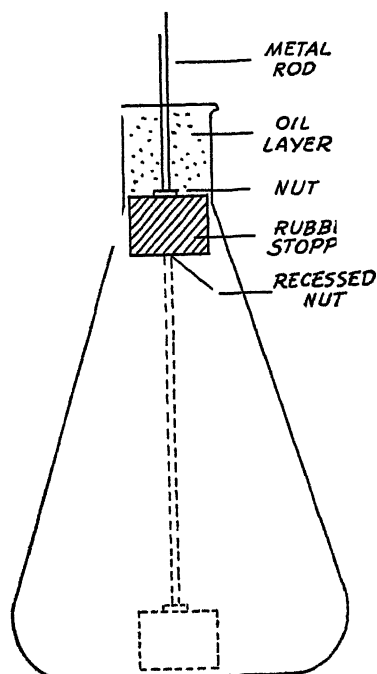


Fig. 2. Wildman trap flask.

N. Y.) is satisfactory when making examinations at magnifications up to 30 diameters. When making examinations at magnifications of 30-40 diameters it is more convenient to have the lines closer together. Lines ruled with oil, alcohol, and waterproof ink are most satisfactory, but for ordinary work a suitable paper can be provided by ruling a smooth-surfaced qualitative filter paper with a sharp-pointed medium-hard black lead pencil. Filter papers of 12.5 cm diameter are most suitable for use where considerable material is liable to be carried onto the fields.



- (e) Plates for holding filter papers for microscopic examinations—A convenient plate can be made by cementing a square piece of light plate glass approximately 3" × 3" onto a piece of thin, smooth asbestos board of the same dimensions.
- (f) Wet pads and covers—Convenient pad for keeping filter papers wet until examinations are made can be provided by placing a smoothly folded towel on a table or glass plate and saturating with water. Filter papers to be examined should be placed on a wet pad and covered with a suitable cover to prevent dirt from settling upon them.
- (g) Microscope—Greenough-type binocular microscopes which afford reasonably wide fields and magnifications of 20–40 diameters for making routine examinations, and higher magnifications of 75–100 diameters to aid in making identifications of small objects, are most convenient for use in examining fields such as those obtained when flotation separations are made from flour and other food products.
- (h) Illuminator—The most suitable light is provided by a low-voltage bulb set in an adjustable mount in an elliptical reflector so that light of suitable intensity can be focused directly into the field from one direction. The light should be mounted on a jointed arm to permit focusing from all angles. A low-voltage lamp provided with adjustable transformer permits the adjustment of light intensity to the requirements of the observer and the work. The intensity of the light must be changed frequently depending upon the color of the objects being investigated, color of the background, and the magnification at which the examination is being made.
- (i) Dissecting needles.

## REAGENTS

All water and reagents should be filtered or examined to insure that they contain no extraneous materials.

- (a) Pancreatin U.S.P.
- (b) Hydrochloric acid. Concentrated C.P.
- (c) Gasoline. Maximum boiling point 130°C.
- (d) Mineral oil. Light grade, white petroleum or paraffin oil
- (e) Alcohol. Ethyl or isopropyl may be used for washing field or breaking emulsions where required.
- (f) Oil solvents. Benzol, petroleum ether, or gasoline may be required at times to aid in dissolving the mineral oil and washing it from the equipment and filter paper.

## GENERAL INFORMATION

Flour from which small particles of extraneous materials are to be recovered should be thoroughly mixed with water and the viscosity reduced to a minimum by heating and digesting with pancreatin or dilute hydrochloric acid.

Gentle stirring is advised to aid in loosening insect fragments, hairs, etc., but violent agitation which may disintegrate fragments and filth particles should be avoided. Violent shaking of digested mixtures with gasoline or oil may also cause emulsions which will hold large amounts of flour and carry it onto the paper.

Pancreatin seems to be the most suitable enzyme for digesting flour and cereal products as its action is fast on both carbohydrates and proteins. The optimum temperature for its action is 40°C. The optimum pH of pancreatic amylase is 6.7-7.2 and pancreatic trypsin 7.8. Unless the flour to be examined is chemically treated, it is not usually necessary to adjust the pH of the cooked water-flour mixture. Pancreatin has little or no effect on insect fragments or hairs.

Insect fragments, hairs, and the usual light particles of extraneous materials are not noticeably affected when boiled with concentrations of 2-3% hydrochloric acid for a reasonable length of time.

Experience is necessary in identifying insect parts, hairs, and other extraneous materials under the microscope. This experience can best be gained by examining known specimens at the magnifications and under the conditions that extraneous materials which are separated from food products are likely to be found.

Only fragments of filth which can be definitely identified should be reported as such. Comments as to doubtful materials should be made. A uniform type of report should be kept by the analyst which will give an adequate description of extraneous materials found, and the quantity of sample examined.

## PROCEDURE

**Section I. Preliminary Examination for Large Elements of Extraneous Materials.** Examine closely around container seams and bag-ties, especially where covered, and where dust has accumulated. If the material is finely ground, smooth out a thin layer with a flour slick or some flat instrument and examine the surface carefully. If the product will pass a 10 mesh U. S. sieve, scoop up portions from the surface and around the edges of the container, and screen. Examine for larvae, insects, clumps of webbing, insect and rodent excreta pellets, and other extraneous materials.

**Section II. Digestion of Sample and Separation of Light Extraneous Materials.** Digestion of the flour sample may be accomplished by either pancreatin or hydrochloric acid.

*Part A. Pancreatin Digestion.* Weigh charge (28–50 g flour) and transfer it to a liter beaker. Add 400 ml water and mix until a smooth suspension free from lumps is obtained. Heat the suspension very carefully with continuous stirring over a gas burner on an asbestos-centered wire gauze until it boils, or in a boiling water bath with stirring until the temperature reaches 85°C and continue heating for about 15 minutes. Cool to 40°–45°C. Add 0.5–1.0 g pancreatin by sprinkling the powder over the surface and stirring it through the suspension. Wash the adhering paste from the sides of the beaker back into the suspension. Allow to digest at a temperature of 35°–45°C until the viscosity of the mixture is reduced to a minimum and the insoluble materials settle readily. This usually takes 3 to 6 hours. Digestions may continue for much longer periods of time without harmful effects on white flours. It is sometimes advantageous to allow digestion to continue overnight, but when digesting graham or whole wheat flours which contain much bran, more satisfactory flotation separations of the light extraneous materials can be obtained after digestions of only 2 to 3 hours. If the digestions continue for longer periods of time, the light bran is loosened and when the flotation separations are made, it is carried onto the fields to be examined along with the light extraneous materials. The bran blocks the fields and makes it difficult to find the insect fragments, hairs, etc.

The gasoline flotation separation of the digested mixture may be accomplished by means of a separatory funnel or a Wildman trap flask as described below.

**USE OF THE SPECIAL SEPARATORY FUNNEL.** The separatory funnel should be a long narrow type, with a short stem having a fairly large bore (Figure 1). This shape is preferred because coarse materials can be mixed with the gasoline or oil and drawn off while maintaining as much distance as possible between the oil-water interface and the outlet. A long heavy wire or thin glass rod may be used to dislodge coarse materials should the stem become clogged. If the mixture contains particles too coarse to pass through the funnel, the water mixture should be mixed thoroughly with the gasoline or oil in the beaker, and the liquid portion decanted into the funnel.

Transfer the mixture obtained by pancreatin digestion from the beaker to the separatory funnel and add enough water to bring the volume up to about 800 ml. Add 25–30 ml of gasoline (max. b.p. 130°C). Stopper the funnel and roll gently in a horizontal position for 3 to 4 minutes. Invert the funnel a few times and release the pressure by

slightly opening the pinch clamp. After thorough mixing to bring the gasoline into contact with all particles, place the funnel in a stand and allow the materials to separate and settle for 5 to 10 minutes, if making separations from white flour. When making separations from graham, whole wheat, and coarse flours, it is advisable to draw off the coarse materials into a large beaker as they settle and decant liquid portions back into the funnel until all the coarse materials which settle have been removed from the funnel. This prevents packing and closing the funnel and also aids in loosening small fragments and hairs which tend to adhere to the coarse particles of cereal.

After settling, draw off several 200-ml portions to within 3 to 4 inches of the gasoline-water interface and decant the liquid portions back into the funnel. Wash all materials down from the inside of the separatory funnel and wash two or three times with 50-100 ml portions of water. Transfer the materials at the interface to a lined filter paper to be described later.

The digested mixture and washings from the first separation should be saved for further flotation separations. For the usual routine examination, a second gasoline flotation separation is advisable. For very thorough work, a third and fourth flotation may be necessary, but unless the product is unusually heavily contaminated, two careful flotation separations should be sufficient.

**OPERATION OF THE WILDMAN TRAP FLASK.** If the Wildman trap flask (Figure 2) is used in making the gasoline flotation separations, transfer the digested water-flour mixture to the flask, or add the flour and water and carry out the digestion in the flask. Add 25-30 ml gasoline, when it is to be used as the flotation medium, and stir it thoroughly through the mixture. Care must be taken that a frothy emulsion is not created which will entrap much flour. Fill the flask with water until the oil layer is brought into the neck so that when the stopper is raised until it closes off the neck of the flask, it will be about 1 cm below the oil layer and floating materials. Stir the contents of the flask gently to loosen light extraneous materials and allow to settle for 10-15 minutes. Spin stopper carefully to remove adhering flour before raising it. Raise the stopper until it closes off the bottom of the neck of the flask and decant the trapped-off oil layer and adhering materials into a 250 ml beaker. Rinse any adhering materials from the rod and neck of the flask into the beaker with alcohol and then water. Another portion of gasoline should be added and the contents of the flask again stirred thoroughly so that any light materials which may have been caught during settling and adhering to the surface of the flask may be loosened and recovered. Add water to

bring the oil layer up into the neck of the flask, allow to settle, trap off, and wash as directed for the first separation.

Where mineral oil is to be used as the flotation medium a second separation should be made using gasoline. The gasoline does not adhere so readily to the rod and sides of the flask and tends to loosen the adhering materials better than the mineral oil.

If the materials trapped off into the beaker are noticeably emulsified, add about an equal volume of ethyl or isopropyl alcohol. The contents of the beaker are now ready to be filtered.

*Part B. Acid Digestion.* Weigh charge (28–100 g flour—as much as 200 g of patent flours may be used), and transfer to a liter beaker. Add 400 ml dilute hydrochloric acid solution (20 ml concentrated 36.5% HCl to 380 ml water), and stir until the flour is thoroughly mixed with the solution. Heat carefully until the mixture begins to boil. Add 20 ml light mineral oil and continue the boiling for 15–20 minutes. A stirring rod should be placed in the beaker to prevent the boiling mixture from bumping and spattering. Cool to room temperature and carry out the mineral oil flotation separation in either the separatory funnel or in the Wildman trap flask as already described.

All materials sticking to the beaker should be carefully rubbed loose and washed into the funnel or flask.

After separating the oil layer by either the separatory funnel or the flask it is ready for filtering.

**Section III. Filtration and Examination.** Draw the oil contents of the funnel or flask onto a filter paper 12.5 cm diameter which has previously been moistened and fitted into a filter funnel. Mild suction should be used in filtering and care should be taken to obtain an even, and not too dense, distribution of the materials over the paper to be examined. When making flotation separations on flour containing considerable bran and germ, some of the light fragments are often held at the oil-water interface and carried onto the filter paper. For this reason paper of 12.5 cm diameter is recommended and can be readily examined under a wide field binocular microscope when cut into quarter sections. If the oil and water holding the entrapped materials which are to be drawn onto the filter paper are noticeably emulsified, the addition of an equal volume of ethyl or isopropyl alcohol will usually break the emulsion and prevent it from clogging the filter. Mineral oil may be washed through the paper with an oil solvent.

*Microscopic Examination of Filter Paper.* Place the wet, lined filter paper containing the separated materials on a flat plate and examine by moving along in the field of vision under the microscope.

For making routine examinations 25–40 diameters should be used. Higher magnifications of 75–100 diameters are convenient and should be used in making identifications of small objects. The entire filter paper containing the separated materials should be examined.

A fine sharp dissecting needle should be used to move particles around in the field of vision. The appearance from various angles and the consistency will greatly aid the analyst in identifying particles of materials.

### Results

Members of the committee and collaborators examined two samples each of white and whole wheat flours. The samples contained considerable amounts of insect fragments and a few rodent hairs of the size which would be found in a flour sample milled from contaminated wheat. After examining the first samples by a number of procedures, the committee decided that most satisfactory results were obtained by the pancreatin-gasoline and acid-mineral oil procedures essentially as described above. A second sample each of white and whole wheat flour was then examined following these procedures and also by procedure M2B, as given in Food and Drug Circular No. 1. Results of these examinations are listed in Table I.

Comparable results were obtained by both procedures on examination of white flour. The hydrochloric acid digestion–mineral, oil flotation separation is the more rapid of the two procedures and probably the more suitable for most control work. Collaborative results showed that the pancreatin digestion–gasoline flotation separation procedure is more applicable to whole wheat flours. Too much bran and germ is carried onto the field by the hydrochloric acid digestion–mineral oil procedure.

Agreement among collaborators on the type of extraneous materials found was satisfactory. Actual counts varied considerably but in general the agreement between analysts who were experienced in making such examinations was satisfactory. A determination of extraneous materials by microscopic examination is not quantitative to the degree of a quantitative chemical determination. Factors such as non-uniformity of samples and the ability of the analyst to recognize small particles of extraneous materials make it almost impossible to obtain strict checks. Quantitative results, however, are not actually necessary to differentiate between a clean flour and a flour which is contaminated.

### Summary

Two simplified procedures for the examination of wheat flours for extraneous materials are described. In one, the flour is digested with

TABLE I

COLLABORATORS' RESULTS FOR EXTRANEEOUS MATERIALS IN FLOUR BY THREE METHODS (RESULTS REPORTED ON POUND BASIS)

Collaborator	Extraneous materials	White flour			Graham flour		
		Procedure			Procedure		
		M2B	Pan-creatin—gasoline	Acid—mineral oil	M2B	Pan-creatin—gasoline	Acid—mineral oil
1	Insect fragments	54	110	200	126	96	112
	Rodent hairs	0	9	5	9	0	0
2	Insect fragments	191	378	351	85 <sup>1</sup>	279	—
	Rodent hairs	0	0	0	0	0	—
3	Insect fragments	—	656	390	Could not make count <sup>1</sup>	976	517 <sup>1</sup>
	Rodent hairs	—	0	9		0	0
4	Insect fragments	182	290	291	218 <sup>1</sup>	309	127 <sup>1</sup>
	Rodent hairs	0	4	0	9	9	9
5	Insect fragments	355	200	251	36	46	82
	Rodent hairs	4	0	0	4	0	0
6	Insect fragments	—	—	452	—	—	—
	Rodent hairs	—	—	18	—	—	—
7	Insect fragments	36	80	32	0 <sup>1</sup>	0 <sup>1</sup>	16
	Rodent hairs	0	0	16	0	0	0
8	Insect fragments	48	32	96	80	32	160
	Rodent hairs	0	0	0	0	0	16
9	Insect fragments	279	360	315	—	198	180
	Rodent hairs	0	0	0	—	0	0
10	Insect fragments	448	464	358	416	736	64 <sup>1</sup>
	Rodent hairs	0	16	0	0	0	0
11	Insect fragments	—	486	855	No count <sup>1</sup>	315	No count <sup>1</sup>
	Rodent hairs	—	36	9		18	

<sup>1</sup> Collaborator reported that the field contained entirely too much bran and germ to be examined satisfactorily.

pancreatin solution after which the extraneous material is separated by flotation with gasoline employing a separatory funnel or a Wildman trap. In the other, the flour is digested with hydrochloric acid solution and a light mineral oil used for the flotation. The extraneous materials are collected on filter paper and examined microscopically.

In a collaborative study, both procedures gave comparable results with a sample of white flour; hydrochloric acid digestion and flotation with mineral oil was the more rapid. With a sample of whole wheat flour, digestion with pancreatin and flotation with gasoline was more

satisfactory than the hydrochloric acid-mineral oil procedure, as the mineral oil flotation tended to trap considerable bran and produce fields which were difficult to examine.

Although the actual counts reported by different collaborators varied considerably, agreement on the types of extraneous materials was satisfactory.

#### Acknowledgments

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## SOME CHARACTERISTICS OF GLIADIN AND GLUTENIN INDICATED BY DISPERSION AND VISCOSITY<sup>1</sup>

MARK A. BARMORE <sup>2</sup>

(Received for publication July 22, 1946)

Since gluten has been shown by Finney (1943) to be the material responsible for the differences in loaf-volume-producing ability of hard wheats, it would appear that a more thorough knowledge of the composition and properties of gluten is essential to an understanding of this characteristic. The present paper reports some preliminary results in the separation and study of gluten components.

The extensive literature reviewed by Bailey (1944) on methods of obtaining protein fractions and studying their properties attests to the importance of the subject. Haugaard and Johnson (1930), Sandstedt and Blish (1933), McCalla and Rose (1935), Blish (1936), Spencer and McCalla (1938), and McCalla and Gralén (1940, 1942) divided gluten into two or more fractions which differed progressively in solubility, molecular shape or symmetry, and in amide and arginine nitrogen. The evidence available has been interpreted by some of the above investigators as indicating that gluten is made up of relatively few distinct protein components and by McCalla and associates as showing that "the main gluten protein is a single complex that can be divided into many fractions differing systematically in both physical and

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

<sup>2</sup> Chemist, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.



chemical properties." Haugaard and Johnson (1930) believe that the protein complex may change reversibly depending on conditions of temperature, salt content, hydrogen ion concentration, etc. Blish (1936), however, has found contradictory evidence supporting the existence of a single protein complex and a mixture of a few distinct individual proteins.

Much of the earlier work with protein separates dealt with the aqueous-alcohol-soluble fraction, gliadin. Gliadins differing in solubility, viscosity, and chemical composition were obtained by Cook (1931) using different methods of preparation and by Haugaard and Johnson (1930) using fractionation. The latter also found differences in optical activity. Sinclair and Gortner (1933) could not confirm Haugaard's and Johnson's differences in chemical composition, however. Differences in molecular weight of gliadin fractions were observed by Krejci and Svedberg (1935).

Viscosity measurements have been used to a limited extent in the characterization of gluten fractions, especially gliadin, and these correlated with solubility. The fact that there has been reported no systematic application of viscosity to all fractions of gluten is doubtless at least partly due to the difficulty in interpreting the data until recent years. More recently, however, Lauffer (1942) has summarized the information available and showed that the rate of change of viscosity with concentration of solute is related to the ratio of molecular length to thickness (axial ratio or symmetry) and that molecular hydration is a minor factor. Since rate of change in viscosity to concentration is related to the absolute viscosity at a given concentration, it follows that solutions of similar material at equal concentrations but different viscosity indicate a difference in axial ratio of the solute molecules. Thus viscosity data can be used to characterize gluten fractions by indicating average molecular or aggregate shape.

### Materials and Methods

The flours used were obtained from composites of pure wheat varieties ground in an experimental mill. The wheat had been grown at various agricultural experiment stations in Kansas, Nebraska, and Colorado. Unless otherwise stated, the dispersions were obtained from gluten which was washed in the 6.8 pH, 0.1% phosphate buffer solution of Dill and Alsberg (1924). Viscosity measurements were made with Ostwald pipettes at 30°C, standardized with water, 20%, and 40% sucrose. The two dispersion media were 50% alcohol and 50% acetic acid. These were made up by volume rather than by weight.

Critical peptization temperature (C.P.T.) was described by Dill and Alsberg (1924) and as used in the present study is the temperature

at which a 2% protein solution in 50% alcohol showed marked opaqueness on slow cooling.

The method of purifying gliadin outlined by Dill and Alsberg (1924) consists essentially of two procedures which will be referred to as "cooling" and "foam" precipitations. The cooling precipitation as used consists of storing an alcoholic 2% protein solution at 4°C for 48 hours. During this time a viscous, honeylike layer formed at the bottom of the flask leaving a cloudy solution which became sparkling clear at room temperature. A double cooling precipitation is one cooling precipitation followed by another applied to a 2% protein solution of the first "honey" layer. The foam precipitation consisted of diluting slowly and shaking vigorously a 2% protein aqueous alcohol solution with five volumes of water containing 0.1% NaCl. Part of the protein was precipitated in the foam and part dissolved in the cloudy solution. The protein in the foam was redissolved readily by adding 95% alcohol. Evaporation of the protein solution was necessary, and was found not to affect resolution or viscosity if the temperature was kept below 40°C. Solutions containing salt were dialyzed and redissolved before viscosity measurements were made. Viscosity values were determined at 2% protein in 50% alcohol unless otherwise indicated. Viscosity measurements for calculations of axial ratio were made on dispersions containing 0.05 to 0.075 *M* NaCl to minimize the electroviscous effect as pointed out by Cohn and Edsall (1943).

## Results

Aqueous alcohol extracts of gluten from different samples of hard winter wheat were found to have similar viscosities, but samples of gliadin purified by slightly different methods were widely different in viscosity even for the same samples. Conversely, preparations by the alternate cooling and foam precipitation method of Dill and Alsberg (1924) produced gliadin of the same viscosity from flour samples known to differ widely in protein quality.

A qualitative study of the fractions resulting from cooling and foam precipitations, as diagrammed in Figure 1, led to information which explained the above results. Two successive extractions of gluten were combined and subjected to a double cooling precipitation. The combined clear solutions contained 26% of the protein, which had a viscosity of 2.74 centipoises. The "honey" layer contained the remainder, or 74%, and had a viscosity of 3.03 centipoises. A foam precipitation separated the honey layer into a soluble portion with a viscosity of 2.87 centipoises containing 24% of the original protein and the foam sediment with a viscosity of 3.09 centipoises containing 43% of the original protein. After finding that a cooling precipitation would not

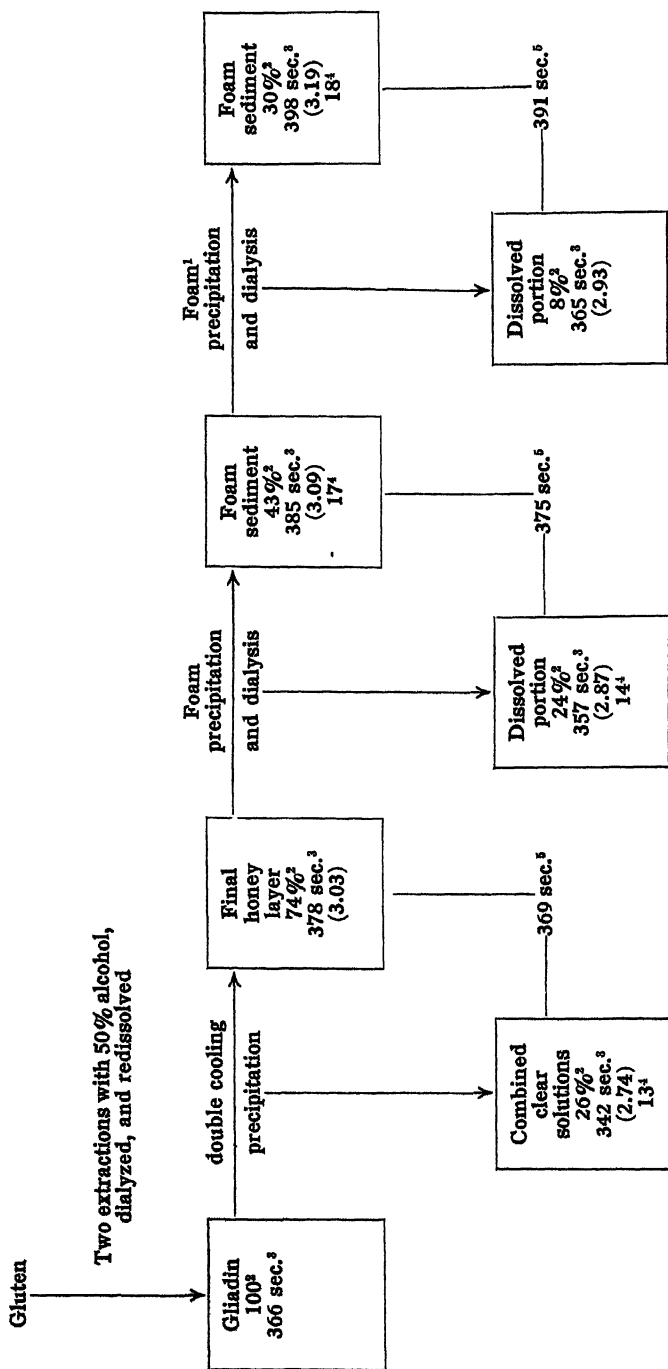


Fig. 1. Schematic plan used in fractionating gliadin.

<sup>1</sup> After finding that a cooling precipitation was not capable of further fractionation.

<sup>2</sup> Values expressed are the amounts of original gliadin in the particular fraction.

<sup>3</sup> Viscosities of 2% protein solutions measured in seconds. Those values in parentheses are the same values given in centipoise<sup>4</sup>.

<sup>4</sup> Calculated axial ratio.

<sup>5</sup> Calculated viscosity of a mixture of the two fractions in original proportions.

fractionate the foam sediment further, it was subjected to another foam precipitation. The resulting soluble portion and foam sediment contained 8% and 30% of the original protein and had viscosities of 2.93 and 3.19 centipoises, respectively.

Since previous work had shown that the viscosity of a mixture of two gliadin fractions was approximately proportional to the relative amounts and viscosity of each fraction, the viscosities of the mixtures of the above fractions in their original proportions were calculated and are given in Figure 1. The agreement of these values with those of the original fractions shows that there was no change in the first four fractions and very little if any in the last two.

The results demonstrate that both the cooling and foam precipitations separate gliadin into fractions differing in solubility and in viscosity. It is easy to understand how gliadins of different viscosity could be prepared from the same original alcoholic extract of gluten by varying the order or number of foam and cooling precipitations, the fractions discarded, the conditions of concentration, temperature, or salt content. Likewise by rigid adherence to procedure and conditions it is possible to prepare gliadin of identical viscosity from gluten extracts that differ considerably.

In the Dill and Alsberg method of purifying gliadin, the high viscosity material is discarded in the cooling precipitation but saved in the foam precipitation. Therefore the final material is a gliadin of medium viscosity which has been separated from other gliadins of both higher and lower viscosity.

The application of Simha's equation (1940) for rodlike ellipsoids to rate of change of viscosity with concentration as outlined by Lauffer (1942) indicates that the molecules of the above fractions with viscosities of 2.74, 2.87, 3.09, and 3.19 centipoises, compared to 2.04 centipoises for 50% alcohol, are, respectively, 13, 14, 17, and 18 times as long as thick. In comparison, Neurath (1939) and Mehl, Oncley, and Simha (1940) obtained axial ratios ranging from 10.5 to 11.1 for purified gliadin calculated from viscosity, sedimentation, and diffusion data. The solubility of these fractions thus appears to be related to the axial ratio or molecular symmetry, the more symmetrical molecules being the more soluble in dilute salt solutions and cold 50% alcohol.

Further evidence of the heterogeneity of gliadins purified by various methods was obtained by determining the critical peptization temperature (C.P.T.) of a number of fractions. In the fractions known to be *fairly* homogeneous as the result of a series of cooling and foam precipitations, the C.P.T. was closely related to viscosity, that is high C.P.T. with high viscosity. This relationship did not hold for relatively unfractionated gliadin solutions. This lack of agreement appeared to be

due to the fact that in this case the C.P.T. indicates the insolubility of only a small portion of the total protein present. Since viscosity expresses the average of all the proteins present, C.P.T. and viscosity would be correlated in the more homogeneous mixtures only.

Since the results obtained by cooling and foam precipitations and the critical peptization temperature study indicate that the aqueous alcohol-soluble protein, gliadin, is heterogeneous, it might be expected that extraction of flour at different temperatures would produce gliadin of varying solubility and viscosity. Accordingly an experiment was set up in which 50-g subsamples of flour were extracted four times with 200 cc of 50% alcohol at  $-12^{\circ}$ ,  $4^{\circ}$ ,  $27^{\circ}$ , and  $60^{\circ}\text{C}$ . Centrifuging or filtering in between extractions was also carried out at these temperatures. At the three lower temperatures agitation was continuous and the extracts were centrifuged. At  $60^{\circ}\text{C}$  agitation was intermittent and the extracts were filtered. The amount of protein obtained by each extraction decreased progressively and in the fourth the flour seemed to be nearly exhausted of soluble protein. Since some of the material extracted at  $60^{\circ}\text{C}$  was not soluble in aqueous alcohol at  $30^{\circ}\text{C}$ , another solvent was required. Since both gliadin and the simpler glutenin proteins could be dispersed in 50% acetic acid, the extracted proteins were dispersed in this solvent for comparison of viscosities. Although proteins hydrolyze slowly in this dispersion medium (Cook and Rose, 1935), the amount of change in viscosity is slight in the time required to complete the analysis and viscosity determinations. It was found that the viscosity of alcohol-soluble flour proteins in 50% acetic acid was a linear function of that in 50% alcohol with a correlation coefficient of  $+0.95$  for 30 pairs.

The results of the extraction are plotted in Figure 2 in which the percent of the total protein obtained by the four extractions and the viscosity of the 2% protein solutions in 50% acetic acid, after dialysis and evaporation, may be compared to the temperature of extraction. Solubility at  $60^{\circ}\text{C}$  or higher temperatures is doubtless limited, owing to denaturation which probably takes place in the more complex molecules. Electroviscous effects were not minimized in these dispersions, and therefore axial ratios have not been calculated from these data.

The data show how the amount as well as the viscosity of the protein dissolved or dispersed is dependent on the temperature. Since viscosity is indicative of the symmetry of the molecules, the less symmetrical the molecule the less is its dispersibility. By common usage gliadin and glutenin are considered to be the major protein constituents—i.e. the aqueous alcohol, room temperature soluble and insoluble fractions, respectively. However, by increasing the temperature to  $60^{\circ}\text{C}$ , about one fourth of the glutenin is dispersible. The increase in

viscosity of the 60°C extract compared with that obtained at 27°C indicates that this soluble glutenin is less symmetrical than the gliadin fraction.

Whole gluten will disperse slowly in 50% acetic acid, forming a smooth dispersion, provided it is agitated and centrifuged. About 10 to 15% of the protein is generally not dispersed sufficiently to be included in the centrifugate. Viscosity measurements can be made on these dispersions although they are not satisfactorily reproducible from one dispersion to another and they show some plastic flow. Hydrolysis as measured by viscosity changes is not detectable if the

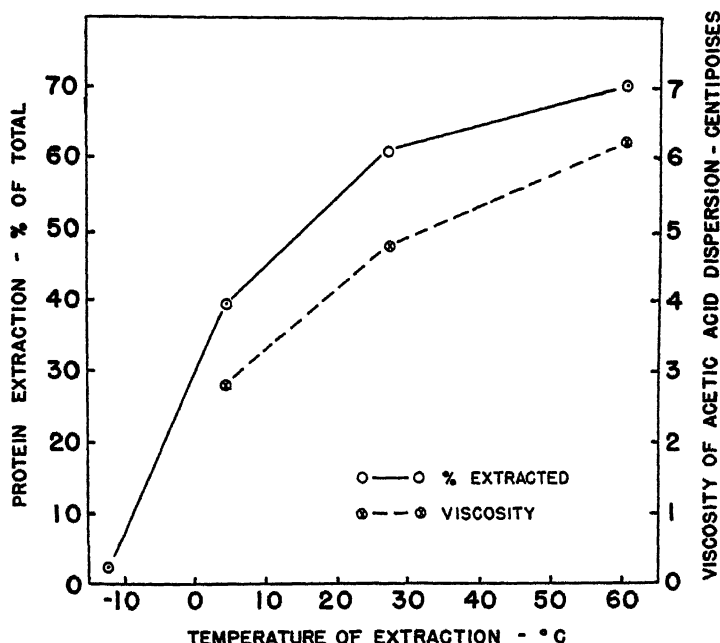


Fig. 2. Amount and viscosity of protein extracted from flour at various temperatures.

dispersion is stored at  $-12^{\circ}\text{C}$  during the dispersion period. In general the viscosity of such gluten dispersion is about 10.5 centipoises, compared to 1.62 centipoises for 50% acetic acid, which corresponds to an average axial ratio of 41. Since the viscosity of a mixture of two protein dispersions is approximately proportional to their viscosities and the amounts mixed, the viscosity of glutenin or the nonalcohol-soluble protein of flour may be approximated. On the basis of 50% of the flour protein being gliadin of axial ratio of 19, the glutenin would have an axial ratio of 47. This adds additional evidence to that of the extraction experiment that the protein insoluble in the 50% alcohol is more complex than the soluble portion.

### Discussion

In calculating axial ratios it was necessary to assume that the shape of the gluten protein molecules was rodlike ellipsoids of revolution, as is pointed out by Lauffer (1942) for gliadin, rather than platelike ellipsoids. pH has been assumed to be of no consequence since the addition of electrolytes reduces the charge on the micellae to such an extent that it does not introduce an important error in viscosity (Lauffer, 1946). It is assumed that hydration plays an unimportant role, compared to molecular shape, in the viscosity of these dispersions (Lauffer, 1942), and it has therefore been left out of the calculations. The specific volume of 0.73 cc used throughout is an average of that obtained by McCalla and Gralén (1942) for gluten proteins in acetic acid and alcohol solutions.

These assumptions and approximations may affect somewhat the accuracy of the values obtained; but since the fractions examined are

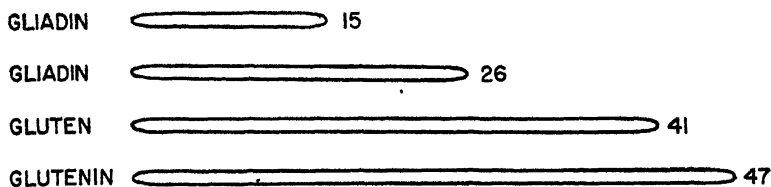


Fig. 3. Proportionate length vs. thickness of various gluten fractions according to results.

still heterogeneous and indefinite and could be duplicated only by rigid adherence to the procedure of preparation, the absolute values of axial ratios are unimportant compared to the relative values.

The dispersions contained some materials other than protein, particularly the gluten dispersions in acetic acid. The main impurity was very finely divided starch that could not be thrown down by centrifuging. However, since the viscosity of these dispersions was high, the amount of starch present did not contribute an appreciable error relative to viscosity. The solutions of the aqueous alcohol-soluble proteins were considerably purer, on the basis of their nitrogen content, and did not contain suspended starch and only a few hundredths of a percent of nonprotein material.

As illustrated in Figure 3 the shape of the wheat protein molecule appears to vary from relatively short ellipsoids, which are about 15 times as long as thick, to the relatively long ellipsoids, which appear to be about 47 times as long as thick (based on the data from the acetic acid dispersions). As the molecular length relative to thickness increases from approximately 15 to 26, the temperature of 50% aqueous

alcohol has to be increased above 4°C in order to get solubility. Increases in temperature above 27°C will dissolve protein of slightly longer relative molecular length. Still longer molecules will disperse in 50% acetic acid, as do the short ones, but there appears to be a small percentage of molecules too complex to disperse even in acetic acid unless complexity is reduced by allowing time for hydrolysis.

Viscosity data alone do not indicate whether the increase in axial ratio is due to a difference in molecular weight or to a lengthening of the molecules of equal molecular weight. Changes in axial ratio could also be due to aggregation of simpler molecules. However, no gliadin seems to be formed in the slow hydrolysis of glutenin by acetic acid, which is some evidence that these are not gliadin aggregates. McCalla and Gralén (1942) have shown that the molecular weight of the gluten protein varies with solubility, and since solubility and viscosity are highly related the increase in axial ratio may be due, at least in part, to an increase in molecular weight.

It seems reasonable to assume that the solubility in sodium salicylate found by McCalla and Rose (1935) parallels the solubility in aqueous alcohol and acetic acid. If this is the case, as the molecule increases in length it decreases in solubility in three solvents, increases in molecular weight, and except for the most soluble 10–15% it increases in arginine nitrogen and decreases in amide nitrogen.

All fractionation, viscosity, and solubility data obtained in the work reported here support the contention that wheat gluten "is a single protein complex that can be divided into many fractions differing systematically in both physical and chemical properties." However, the possibility of change in molecule size or shape postulated by Sinclair and Gortner (1933), Haugaard and Johnson (1930), and Blish (1936) by treatment with electrolytes, hydrogen ions, precipitation technic, etc., has not been eliminated.

### Summary

Wheat flour gluten has been fractionated into components differing progressively in viscosity and solubility. The differences in viscosity have been interpreted to indicate differences in axial ratio of rodlike ellipsoidal molecules.

The molecules of the protein commonly called gliadin appear to be the most symmetrical and the most soluble. However, some of these molecules appear to be twice as unsymmetrical as others. Glutenin molecules likewise vary in symmetry and are more unsymmetrical than those of gliadin. The more symmetrical glutenin molecules are soluble in hot aqueous alcohol, but insoluble at room temperature. Symmetry and solubility in several solvents appear to be related, the



more symmetrical the molecule the greater the solubility or dispersibility. The data add further evidence to that in the literature that gliadin and glutenin are part of a complex protein system differing systematically in physical and chemical properties with no clear distinction between the two.

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# KINETICS OF BETA-AMYLASE ACTION IN 20% STARCH PASTES AT ELEVATED TEMPERATURES

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The kinetics of amylase action are discussed in various degrees of complexity in the literature (Kneen, 1944), and a good description of elementary principles of hydrolytic enzyme action is given by Van Slyke (1942). The kinetics of diastase action in particular go back to the work of Kjeldahl (1879) and of Lintner (1886), and to a more comprehensive study by Brown and Glendinning (1902) of the saccharifying action of diastase on starch. Wohlgemuth (1908) described the dextrinizing action of diastase on starch. More recent studies have made use of these basic principles to measure the strengths of alpha- and beta-amylases individually (Claus, 1946). In general, however, amylase kinetics have been studied in 2-3% starch pastes, and at temperatures well below those which cause inactivation of the enzymes. But the brewer is interested in what occurs when malt amylases act on starch concentrations approximating those in a brewing mash (about 20%) and at brewing temperatures of 50°-80°C, in the range which causes rapid inactivation of the amylases. This report covers the first phase of investigations on the kinetics of purified malt amylases acting on 20% corn starch pastes at elevated temperatures.

## Apparatus and Procedure

The experiments were carried out on 20% corn starch pastes which had been liquefied with pure alpha-amylase. The enzyme was added to the starch milk which was rotated in a 500-ml mixing flask (an Erlenmeyer with vertical vanes blown into it) in a water bath, and brought up through a definite time-temperature schedule to gelatinize and liquefy the starch. The solution was then boiled 20 minutes, buffered with acetate, and brought to weight with water. The finished substrate was 20.8% starch by weight.

To convert, 48 g of substrate was weighed into a 125-ml mixing flask, which was then rotated by a hollow shaft in a water bath, as shown in Figure 1. The beta-amylase, in 2 ml of solution, was introduced below the surface of the starch with a pipette, and rotation of the flask produced rapid mixing. The resulting mixture was 20% starch.

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From time to time during the conversion, 1.5–2.0 g samples of the mixture were withdrawn directly into weighed 50-ml flasks containing 10 ml of 0.2% sodium hydroxide solution which instantly stopped the enzyme action. The flasks were then reweighed to ascertain the amount of the sample to the nearest milligram. Each withdrawal was made with a clean, dry tube system as shown in Figure 1. Further dilution was then made with the alkali solution, on an analytical balance, so that the diluted solution contained 0.5 g of converted substrate per 5.0 ml of solution. 5.0 ml aliquots were titrated with 10 ml of ferricyanide solution (Hildebrand and McClellan, 1938) and 0.05 *N*

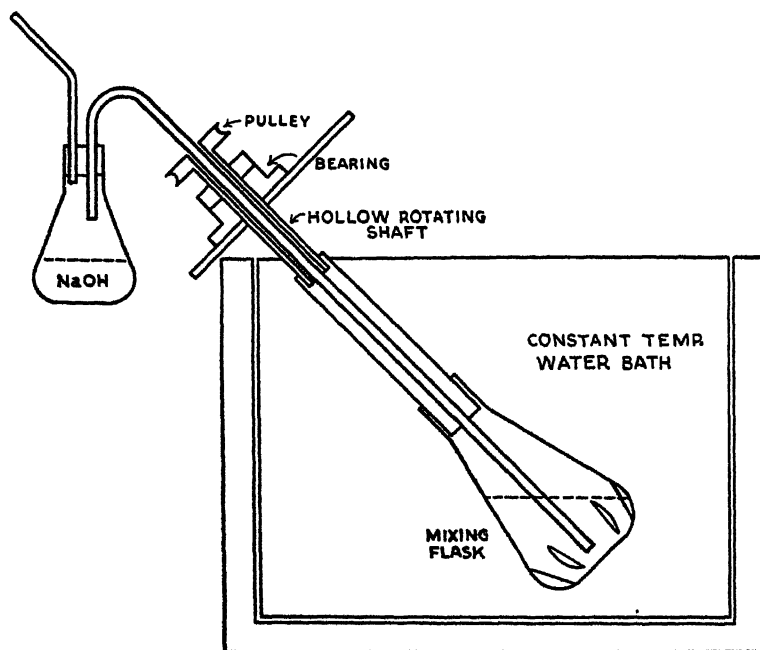


Fig. 1. Schematic diagram of conversion apparatus.

ceric sulfate solution to determine the reducing power of the aliquot. Careful calibration showed that 1.0 ml of 0.05 *N* ceric sulfate solution corresponded to 1.59 mg pure maltose (corrected for hydrate) in the presence of 0.5 g of the substrate.

Titration were carried out serially, one tube being prepared every 4 minutes, heated in a boiling water bath 20 minutes, cooled 4 minutes, then titrated. These titrations were accurate to within a few hundredths of a milliliter of ceric sulfate solution, so that duplicate titrations were rarely carried out. Much more dependable results were obtained by preparing more samples and titrating singly.

### Theoretical Considerations

*The Reciprocity Relationship.* If the reciprocity relationship is valid, the time required for a given amount of enzyme to produce a definite amount of action (such as an arbitrary amount of reducing power) is inversely proportional to the amount of enzyme. To test the validity, conversions were carried out with various small amounts of beta-amylase, and samples were withdrawn very soon after the addition of the enzyme.

Several such experiments were performed, with results consistently like those shown in Figure 2. In the experiment shown, 1.65 and 3.30 K-S units of beta-amylase (Kneen and Sandstedt, 1941) were added

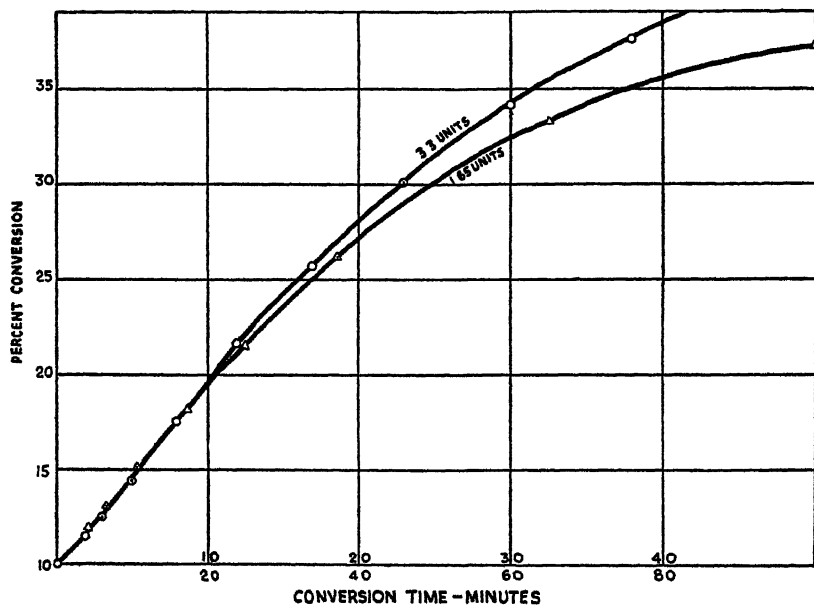


Fig. 2. Conversion of 20% starch paste by 3.30 and 1.65 K-S units of beta-amylase, at 40°C and pH 5.5, showing failure of reciprocal relationship.

to two simultaneous conversions at 40°C. In the figure, the lower time scale is for the smaller amount of enzyme (lower curve), and the upper time scale is for the larger amount of enzyme (upper curve). If the reciprocity relationship were valid for all degrees of conversion, the two curves would be superimposed. Actually, they coincide only up to about 17-18% conversion, including 10% due to liquefaction of the substrate. Within this small range, conversion is proportional to time and to amount of enzyme. That is, it corresponds to a zero-order reaction, and appears to offer the best field for elementary studies of beta-amylase kinetics.

*Determination of "Activity" and "Inactivation Index" from Experimental Data.* Assuming that beta conversion does proceed according to a zero-order reaction, one may write that the increment in the amount of maltose,  $dM$ , in time  $dt$ , is proportional to the amount of active enzyme,  $\beta$ , and to its activity,  $A$ . That is:

$$dM = A\beta dt \quad (1)$$

Suppose that the amount of active enzyme,  $\beta$ , at any time,  $t$ , is the result of logarithmic inactivation by the heat, or that

$$\beta = Be^{-kt} \quad (2)$$

where  $B$  is the amount of enzyme added at the beginning of the experiment (when  $t = 0$ ) and  $k$  is the temperature inactivation index, which may also be thought of as the logarithm of the fraction of active enzyme which is inactivated per unit of time. Substituting (2) in (1) and integrating, we have

$$\int_{M_0}^M dM = \int_0^t AB e^{-kt} dt \quad (3)$$

$$M - M_0 = AB(1 - e^{-kt})/k \quad (4)$$

$M_0$  represents the amount of maltose present when  $t = 0$  (reducing power of the substrate calculated as maltose) and  $M$  is the amount of maltose after conversion for time  $t$ . The difference,  $M - M_0$ , which is the significant value, may be represented as  $\Delta M$ . If the conversion is carried out at a sufficiently low temperature so that no inactivation occurs,  $k$  is zero, and equation (4) becomes:

$$\Delta M = ABt \quad (5)$$

which is the equation of a straight line. Experimentally, it is observed that the conversion curves are straight lines for temperatures below 45°C, in which case the value of activity  $A$  is easily calculated from the slope of the observed curve (Figure 3).

If  $M$  is calculated from experimental data as grams of maltose produced during the conversion, and  $B$  is expressed in terms of K-S units, then the enzyme activity  $A$  will be expressed as grams of maltose per K-S unit per unit of time—minute or hour.

The calculation of  $A$  and  $k$  from experimental data and equation (4) is relatively simple. The value of  $k$  depends on the *shape* of the curve, and  $A$  upon the *amount* of reducing power. In practice, it is simpler to make calculations from experimental data in terms of milliliters of ceric sulfate solution required for titration, milligrams of enzyme used, and minutes of conversion time. Values of  $A$  so calculated can be

changed into whatever units are desired by multiplying by a suitable factor. Calculations are made as follows. Equation (4) takes the form:

$$RP_1 = AB(1 - e^{-kt_1})/k \quad (5a)$$

$$RP_2 = AB(1 - e^{-kt_2})/k \quad (5b)$$

where  $RP$  represents the increase in reducing power in time  $t$ , in terms of ml 0.05  $N$  ceric sulfate solution. It is most convenient to choose values of conversion time such that  $t_2 = 2t_1$ . Then

$$e^{-kt_2} = e^{-2kt_1} = (e^{-kt_1})^2$$

Using this expression and dividing equation (5b) by (5a), one obtains

$$e^{-kt_1} = RP_2/RP_1 - 1 \quad (6)$$

From experimental curves of  $RP$  plotted against time, and use of equation (6), a number of values of  $k$  can be calculated along the course of the curve. The average value of  $k$  is taken as the "temperature inactivation index." From it the "half-life" of the enzyme can be calculated by the formula:

$$\text{Half-life} = 0.692/k \quad (7)$$

In several dozen experiments, the values of  $k$  as calculated from various parts of an observed curve were remarkably constant, indicating beyond doubt that the assumption of logarithmic destruction of the enzyme by heat is valid.

With the value of  $k$  established for any given conversion curve, one substitutes it in equation (5a) and calculates a number of values of  $A$  from various values of  $RP$  on the curve. The average  $A$  is taken as the value for that experiment. Now with known values of  $A$  and  $k$ , one can calculate the entire  $RP-t$  curve, and determine how well it fits the experimental points.

An illustrative set of calculations is given in Table I and shown graphically in Figure 3, in which the solid line represents the calculated curve, and the points are experimental. Especially to be noted in the table is the constancy of the various values of  $A$  and  $k$ , and the excellent agreement between calculated and observed values of reducing power. This one is by no means an exceptional experiment.

### Experimental Results

*Activity and Temperature Inactivation as a Function of pH, at a Conversion Temperature of 65°C.* Values of activity  $A$  and of the temperature inactivation index  $k$  were determined for a number of values of pH between 4.68 and 6.85, all at a conversion temperature

TABLE I

CONVERSION AT 60°C, PH 5.5, WITH 5 MG<sup>1</sup> BETA-AMYLASE

Conv. time (mins.)		RP (ml 0.05 N ceric sulfate)			<i>k</i>	<i>A</i> <sup>5</sup>
<i>t</i> <sub>1</sub>	<i>t</i> <sub>2</sub>	Obs. <sup>2</sup>	Smooth <sup>3</sup>	Calc. <sup>4</sup>		
2	4	0.63	0.63	0.630	—	0.0667
3	6	—	0.92	—	0.0592	—
4	8	1.18	1.18	1.191	0.0568	0.0661
5	10	—	1.45	—	0.0607	—
6	12	1.73	1.69	1.689	0.0584	0.0668
7	14	—	1.92	—	0.0589	—
8	16	2.12	2.12	2.132	0.0564	0.0664
9	18	—	2.33	—	0.0589	—
10	20	2.52	2.52	2.525	0.0602	0.0666
12	—	2.88	2.88	2.875	—	0.0668
14	—	3.24	3.21	3.187	—	0.0672
16	—	3.40	3.47	3.464	—	0.0669
18	—	3.70	3.74	3.710	—	0.0673
20	—	3.95	3.92	3.929	—	0.0666
				Average	0.0587	0.0667 <sup>5</sup>

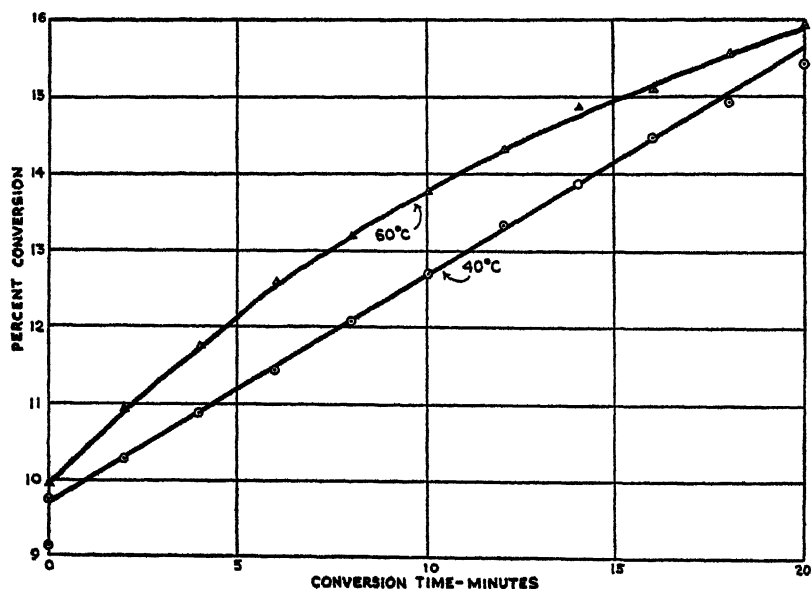
<sup>1</sup> 1.0 mg beta-amylase equivalent to 0.165 K-S units.<sup>2</sup> Experimentally observed RP at conversion time *t*<sub>1</sub>.<sup>3</sup> RP from best smooth curve through experimental points, used to calculate *k* and *A*.<sup>4</sup> RP calculated for *t*<sub>1</sub> using average values of *k* and *A*.<sup>5</sup> *A* in terms of grams maltose per K-S unit per hour = 0.0667 × 57.84 = 3.858.

Fig. 3. Conversion of 20% starch paste by beta-amylase at pH 5.5. Lower curve at 40°C; inactivation  $k = 0.0$ , activity  $A = 1.88$ . Upper curve at 60°C; inactivation  $k = 0.0587$ , activity  $A = 3.858$  (Table I).

of 65°C which is approximately the optimal temperature for our beta-amylase preparations.

The results are summarized in Table II and are graphed in Figure 4. Values of activity  $A$  are given in terms of grams of maltose per K-S unit per hour to show how beta activity under these conditions of substrate concentration, temperature, and pH compare with beta

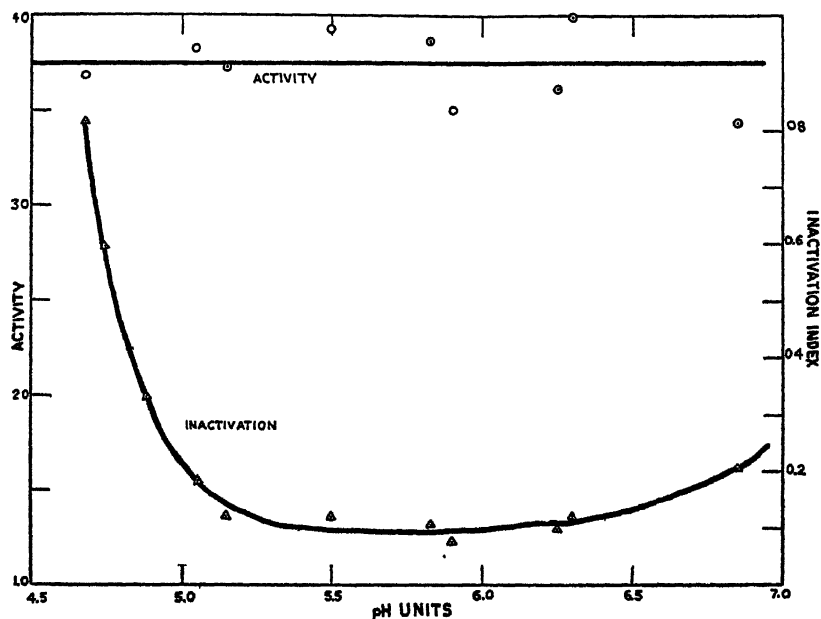


Fig. 4. Activity  $A$  and inactivation  $k$  of beta-amylase in 20% starch paste at 65°C, as a function of pH. Left-hand ordinate scale for activity curve, right-hand ordinate scale for inactivation curve.

TABLE II  
ACTIVITY AND INACTIVATION OF BETA-AMYLASE AT 65° C  
AS A FUNCTION OF PH

pH	Activity <sup>1</sup> $A$	Inactivation <sup>2</sup> $k$	Half-life (mins.)
4.68	3.69	0.810	0.85
4.80	3.52	0.278	2.49
4.90	3.49	0.235	2.94
5.05	3.82	0.180	3.86
5.15	3.73	0.121	5.70
5.50	3.93	0.125	5.55
5.83	3.87	0.106	6.56
5.90	3.50	0.078	8.91
6.25	3.62	0.100	6.92
6.30	4.10	0.123	5.63
6.85	3.44	0.207	3.35

<sup>1</sup> Grams maltose per hour per K-S unit of beta-amylase.

<sup>2</sup> Per minute.



activity under the conditions which define unit activity (K-S unit)—that is, 2% starch solution, 30°C, pH 4.63, resulting in the production of maltose at the rate of one gram per hour per unit of beta-amylase. Values of  $k$  are given in terms of the logarithm of the fraction of enzyme inactivated per minute.

Although the graph shows a certain amount of scatter for the activity values, there is no evidence that activity tends to change with pH in the range from 4.68 to 6.85. The average value is 3.7 times unit activity. On the other hand, the inactivation of the enzyme is quite sensitive to pH outside the broad minimum between about pH 5.4–6.4. Within this range, it may be said that both activity and inactivation rate remain sensibly constant. This range for other temperatures has not been determined.

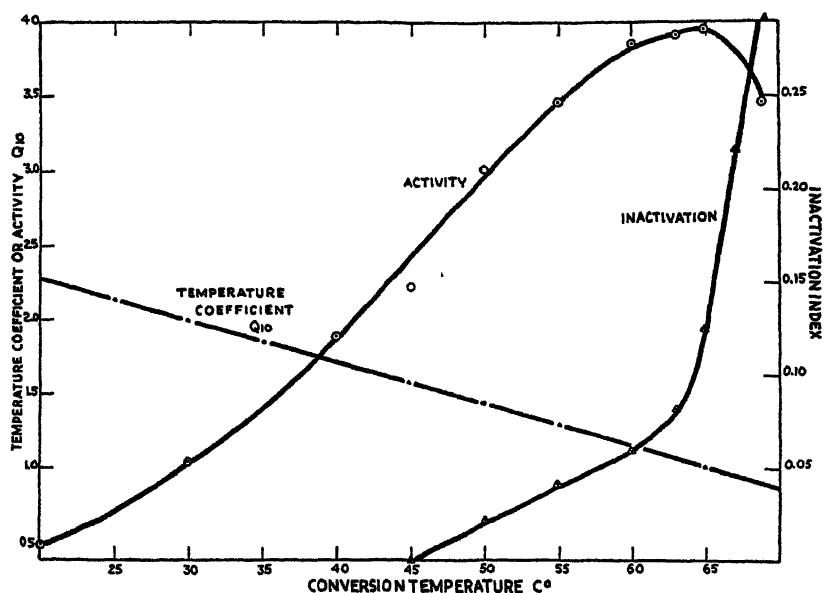


Fig. 5. Activity  $A$ , temperature coefficient  $Q_{10}$ , and inactivation  $k$  of beta-amylase in 20% starch paste of pH 5.5, as a function of conversion temperature. Left-hand ordinate scale for activity and temperature coefficient; right-hand ordinate scale for inactivation.

*Activity and Temperature Inactivation as a Function of Temperature, at pH 5.5.* Values of  $A$  and  $k$  were determined at 11 different conversion temperatures between 20°C and 69°C, all in substrates buffered at pH 5.5. The results are summarized in Table III, and graphed in Figure 5. Values of activity (an intrinsic function of the *live* enzyme, not to be confused with over-all converting power which includes the effects of inactivation) are found to rise to a maximum at about 65°C, and to decrease at higher temperatures. Of interest, though perhaps

fortuitous, is the fact that the activity of one K-S unit of beta-amylase is still 1.0 at 30°C, even though substrate concentration and pH are quite different from standard.

Values of the temperature coefficient of activity,  $Q_{10}$ , are also shown. These values were determined from the activity-temperature curve by drawing a tangent to the curve at the temperature shown, and calculating the ratio of activities indicated by the tangent at temperatures

TABLE III  
ACTIVITY AND INACTIVATION OF BETA-AMYLASE AT pH 5.5  
AS A FUNCTION OF TEMPERATURE

Temp.	Activity <sup>1</sup> <i>A</i>	Temp. coefficient $Q_{10}$	Inactivation <sup>2</sup> <i>k</i>	Half-life (mins.)
20°C	0.49	—	0	Infinitc
25	—	2.14	—	—
30	1.04	2.00	0	Infinite
35	—	1.85	—	—
40	1.88	—	0	Infinite
45	2.22	1.59	0	Infinite
50	2.99	1.46	0.021	32.4
55	3.46	1.29	0.041	17.1
60	3.86	1.15	0.059	11.8
63	3.91	—	0.081	8.6
65	3.93	1.00	0.125	5.6
67	(4.25) <sup>3</sup>	<1	0.221	3.1
69	3.46	<1	0.292	2.4

<sup>1</sup> Grams maltose per hour per K-S unit of beta-amylase.

<sup>2</sup> Per minute.

<sup>3</sup> Obviously high—omitted from Figure 5.

5°C above and below the point of tangency.  $Q_{10}$  as thus calculated is really a *rate* of change of activity per 10°C, rather than a simple ratio of activities at temperatures 10°C apart. These values of  $Q_{10}$  fall remarkably well on a straight line,<sup>1</sup> from a value of 2.14 at 25°C to a value of 1.0 at 65°C, and dropping below 1.0 at higher temperatures.

Inactivation of the enzyme by heat does not take place at pH 5.5 at temperatures below about 45°C. Inactivation occurs above 45°C,

<sup>1</sup> This fact permits determination of the equation of the curve for activity vs. temperature. Values of  $Q_{10}$  as calculated above are given by the expression

$$\left( A + \Delta T \frac{dA}{dT} \right) / \left( A - \Delta T \frac{dA}{dT} \right)$$

where  $\Delta T = 5^\circ\text{C}$  above and below the point of tangency. The equation of the straight line for  $Q_{10}$  vs.  $T$  is

$$Q_{10} = K - cT$$

where  $K$  has the experimental value 2.8525 and  $c$  is 0.0285. Upon integration of the equation

$$\frac{A + \Delta T \frac{dA}{dT}}{A - \Delta T \frac{dA}{dT}} = K - cT$$

one obtains

$$A^{c\Delta T} = C(K + 1 - cT)^{K+1} e^{-(K+1-cT)}$$

$C$  is the constant of integration having the empirical value 2.25. This expression fits the activity-temperature curve of Figure 5 with a high degree of precision, and indicates that it may be theoretically possible to determine activities at all temperatures from a few experimental points at convenient temperatures.

and the curve of  $k$  against temperature shows a sharp increase in the rate of inactivation at  $63^{\circ}$ – $65^{\circ}\text{C}$ , corresponding approximately with the temperature of maximum activity.  $63^{\circ}$ – $65^{\circ}\text{C}$  may therefore be considered as a very critical point in the enzyme-starch reaction at pH 5.5.

*Optimal Conversion Temperatures.* The optimal temperature for a conversion cannot in general be determined except in conjunction with a definite conversion time. It is interesting to calculate, with the help of equation (4) and the values of  $A$  and  $k$  from Table III, what

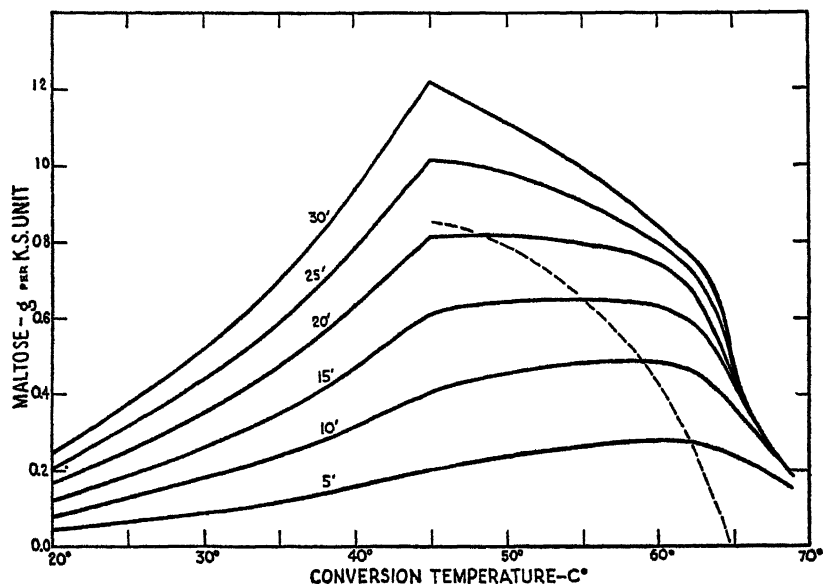


Fig. 6. Saccharification of 20% starch paste of pH 5.5 by beta-amylase, as a function of time and conversion temperature. The dotted line indicates the shift of maximum saccharification toward higher conversion temperatures as conversion time is decreased.

would be the amount of maltose produced per unit of beta-amylase under these conditions, for various conversion times and temperatures. The results are shown graphed in Figure 6. It is seen that for all periods above about 20 minutes,  $45^{\circ}\text{C}$  is the optimal temperature, but for shorter periods during which inactivation occurs and saccharification does not proceed so far, the optimal temperature shifts to higher values to make use of the larger values of  $A$ .

### Summary

Conversion of 20% starch pastes by beta-amylase has been studied at pH 5.5 and at temperatures between  $20^{\circ}\text{C}$  and  $69^{\circ}\text{C}$ ; also at  $65^{\circ}\text{C}$  and at pH values between 4.68 and 6.85.

Under these conditions, production of maltose takes place according to a zero-order reaction up to about 18% conversion. The enzyme is inactivated exponentially by temperatures above 45°C. Mathematical treatment of conversion curves permits determination of the intrinsic activities of the enzyme, and of its temperature inactivation indices.

Activity at 65°C remains constant over the pH range studied, while inactivation is at a minimum between pH 5.4 and 6.4, increasing on either side.

Activity at pH 5.5 increases eight-fold between 20°C and 65°C, at which temperature it reaches a maximum and begins to decrease.

The temperature coefficient  $Q_{10}$  of activity decreases linearly from 2.14 at 25°C to 1.0 at 65°C and to less than 1.0 at higher temperatures.

Temperature inactivation sets in at 45°C and becomes greater as temperature increases. The curve shows a sharp rise at about 63°–65°C, corresponding approximately with the activity maximum.

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# SOURCES OF BETA-AMYLASE AS SUPPLEMENTS TO BARLEY MALTS IN SACCHARIFICATION AND FERMENTATION<sup>1</sup>

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Increase in the use of industrial and beverage alcohols has resulted in the much-increased use of diastatic material other than malt, such as "mold bran." There are some processes where such fungal and bacterial material may not be desirable. Possible nonmicrobial sources which could be used as supplements to malt are sulfite solution extracts of flour (Balls and Tucker, 1943) and sweet potatoes (Balls, Thompson, and Walden, 1946). In contrast to the microbial amylases, these sources are practically devoid of alpha-dextrinogenic activity, the total amylolysis being due to the beta-amylase type of enzyme.

In a study of the alpha- and beta-amylase of malts from many varieties of barley, Nelson and Dickson (1942) found that, in general, high diastatic power is associated with high values in both alpha and beta components, whereas malts of California barley varieties showed very low beta values, but normal to high alpha values. Obviously, this discrepancy may be remedied by the addition of sources rich in beta-amylase such as wheat and sweet potatoes.

Beta-amylase is generally regarded as the component in malt principally responsible for saccharification, but it is also evident that its action is not only facilitated but greatly extended by the presence of alpha-amylase. Thus Kneen, Beckord, and Sandstedt (1941) estimated after comparing the starch-degrading properties of 12 barley malts that over three-quarters of the degree of saccharification obtained was due to beta-amylase. But they also pointed out that several malts high in alpha-amylase showed greater saccharogenic activity than would be predicted on the basis of the beta-amylase content. There was, however, no correlation between alpha-amylase and saccharogenic activity.

The necessity of alpha-amylase for the practical saccharification of starch is obvious from the fact that this enzyme is the characteristic amylase of malted grains. Kneen (1944) found that the ungerminated grains of a number of cereals possess alpha-amylase activity in measurable quantities; but the amount reported is very small compared to that developed during germination. Recently Balls and Schwimmer

<sup>1</sup> Enzyme Research Laboratory Contribution No. 97. Part of this work was done under the Special Research Fund authorized by the Bankhead-Jones Act of June 29, 1935.

(1944) and Schwimmer (1945) have shown that raw starch is completely digested by alpha-amylase of animal origin free from beta-amylase, provided the end products of the reaction are removed. This removal could be accomplished by dialysis or by the action of added maltase. Beta-amylase, on the other hand, has little or no effect on raw starch under similar conditions. The latter enzyme often exists in greater quantities in unmalted than in malted grain, and might perhaps be thought of as essentially the amylase of mature, resting tissue.

Thorne, Emerson, Olson, and Peterson (1945) have presented evidence that amylase determinations are helpful, but not entirely adequate for the evaluation for alcohol production. The latter could probably best be obtained by fermentation tests since the limiting factor in alcohol yield appeared to be the action of the malt rather than the fermenting power of the yeast.

The present communication presents a study of the concomitant saccharifying action of malts and supplementary sources of beta-amylase. The experimental limits within which the saccharifying activity can be expressed linearly and a comparison of calculated and found values for this activity have been investigated. The possible relationship between nonamylolytic enzymes present and discrepancy from calculated values has also been investigated. Finally the effectiveness of these supplements as sugar producers (when used in equi-amylolytic amounts) has been compared with their effectiveness in alcoholic fermentations.

### Methods and Materials

Alpha- and beta-amylase were determined by the methods of Olson, Evans, and Dickson (1944). The definitions used by these workers are based upon "specific" activity rather than "total" activity. Thus the "maltose equivalent" is defined in terms of a definite dry weight of malt. In actual practice this value is calculated as (blank-titration)  $\times 144$ , the titration being that of a 5 ml digestion mixture aliquot (taken from a total of 200 ml) in 10 ml of 0.05 *N* ferricyanide when titrated with 0.05 *N* thiosulfate. Under their conditions this aliquot contains 1.25 mg of malt. For the purposes of the present work it is desirable to transpose this "specific" activity into a unit which does not infer weight of enzyme preparation or source used. This can be accomplished by defining one unit of enzyme as that amount of enzyme which will cause a change in titration of 1/180 ml.

$$\text{Amylase units} = (\text{blank-titration } (\Delta)) \times 180$$

When defined this way, amylase units per mg of (dry) malt become

identical with the maltose equivalent:

$$\text{Maltose equivalent} = \text{amylase units per mg} = \frac{\Delta \times 180}{1.25} = \Delta \times 144$$

Maltase and glucose were determined by the method of Schwimmer (1945), phosphatase by the hydrolysis of nitrophenol phosphate (Axelrod, 1947), and phosphorylase by the method of Green and Stumpf (1942).

Fermentation was carried out according to the procedure of Thorne *et al.* (1945) except that the autoclaved mash was cooled by the addition

TABLE I

AMYLASES, NITROGEN, AND MOISTURE OF MALTS FROM DIFFERENT BARLEY VARIETIES AND OF POSSIBLE SUPPLEMENTARY DIASTATIC SOURCES

Variety or selection	Location grown	Moisture	Nitrogen (dry)	Diastatic power		Maltose equivalent		Ratio
				$\sigma_L$	Maltose equivalent	Beta-amylase	Alpha-amylase	Beta Alpha
M2329 Kindred	Minnesota	8.18	2.33	254	1015	895	120	7.5
A.M. 11 Norwegian	North Dakota	7.93	2.27	208	832	687	145	4.7
A.M. 2 Ezond	Nebraska	8.38	2.79	177	706	650	56	11.6
A.M. 1 Atlas	California	8.40	1.72	68	274	224	50	4.5
Commercial I	Wisconsin	8.54	1.80	93	376	348	28	12.4
Commercial II	Wisconsin	8.48	2.43	175	698	664	34	19.5
Commercial III	California	8.10	1.64	63	252	214	38	5.6
Commercial IV	California	8.39	1.68	81	322	236	86	2.7
Flour <sup>1</sup>	California	12.30	2.85	158	632	632	0.04	—
Flour <sup>2</sup>	California	—	—	43	172	—	—	—
Sweet potato (whole)	California	72.9	0.73	188	750	750	0.03	—
Sweet potato juice (dry)	California	10.43	2.75	1300	5200	5200	5	—

<sup>1</sup> Sulfite extract of flour.

<sup>2</sup> Water extract of flour.

of ice water, while the mash was subjected to the action of a "blendor." Yeast was obtained from the fermentation vats of a nearby distillery.<sup>3</sup>

Four of the eight barley malts used in this investigation were well-characterized barley strains<sup>2</sup> and four were commercial malt samples, two of which were California malts and the other two Wisconsin malts (Table I).

Samples of high protein patent flour and market sweet potatoes were used as supplementary diastase sources. For the study of saccharifying activities, a 10% flour suspension in 0.05% NaHSO<sub>3</sub> was incubated at 30° for 1 hour, and then centrifuged, the residue being

<sup>3</sup> We wish to thank D. F. Logan of the Hedgeside Distillery, Napa, California, for generous supplies of yeasts and malts, and Dr. A. D. Dickson of the Malt and Barley Laboratory, University of Wisconsin, for the samples of well-characterized malt varieties.

discarded. A preparation from sweet potato was made by squeezing mashed sweet potatoes through cheese cloth, centrifuging down the insoluble residue, and drying the resultant juice *in vacuo* while frozen.

In the fermentation tests, hard red winter wheat was used as source of starchy material. As a diastatic supplement, either a suspension of flour in sulfite solution was added to the malt slurry (when flour was used) or homogenized whole sweet potato was used in the fermentation tests (Table I).

### Departure of Starch Conversion from Linearity with Increasing Enzyme Concentration

Comparative experiments on the concomitant action of supplement can be made if one either allows the conversion to proceed in those regions where change in enzyme concentration bears a linear relation-

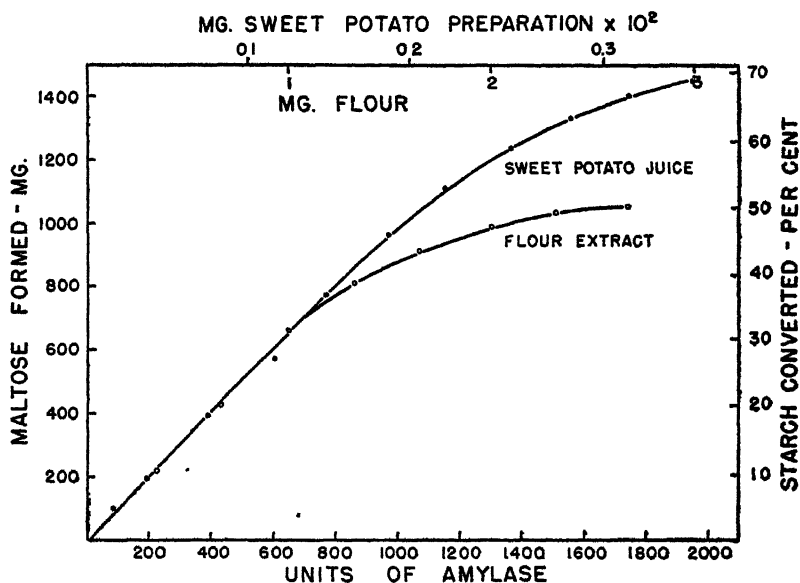


Fig. 1. Relationship between enzyme concentration and extent of starch conversion by sulfite extract of flour (open circles) and dried water-soluble extract of sweet potato juice (closed circles). The enzyme sources are given as mg per 5 ml total digestion mixture. The maltose is that formed from 2 g starch.

ship to sugar formed or if one can determine beforehand the deviation from linearity. The latter procedure is unsatisfactory, for, as can be seen in Figure 1, the deviation from linearity occurs at different stages of digestion for each source of amylase. Thus Kneen and Sandstedt (1941) found that the amylase of wheat shows linear digestion to about 30%, whereas with malt this linearity extends to 40%. This difference, confirmed for wheat in Figure 1, is due to the presence of alpha-amylase



in the malt. Since sweet potatoes contain practically no alpha-dextrinogenic activity (Table I), it is rather surprising to find a linear relationship up to the extent of about 50%, a circumstance which would indicate the participation of nonamylolytic enzymes, possibly of the oligosaccharase or the phosphate transferring classes. Some glucose was found at later stages of conversion for sweet potatoes but not for flour nor for barley malt. Table II consists of a summary of data showing the relative concentrations of maltase, acid phosphatase, and phosphorylase present in wheat and sweet potatoes. It can be seen that wheat contains no maltase but appreciable though small quantities of phosphatase and phosphorylase. On the other hand, the dried sweet potato juice is comparable in maltase strength to takadiastase (Schwimmer, 1945) and is quite high in phosphatase activity.

TABLE II  
CONCENTRATION OF SOME NONAMYLASE ENZYMES IN SWEET POTATO AND IN FLOUR

Enzyme	Flour		Sweet potato	
	Units		Units	
	Per mg (dry)	Per unit of amylase	Per mg (dry)	Per unit of amylase
Maltase	0.00	0.00	0.04	$8 \times 10^{-6}$
Phosphatase	0.3	$4.9 \times 10^{-1}$	25	$48 \times 10^{-1}$
Phosphorylase	$2 \times 10^{-1}$	$3 \times 10^{-7}$	$26 \times 10^{-1}$	$5 \times 10^{-7}$

### Effect of Supplementing Malts on the Saccharogenesis

Figure 1 shows that in order to interpret the results when flour is used as supplement, the enzyme concentration must be kept within a limit such that the total amylase present must not cause a conversion amounting to more than 33%. This corresponds to a titration value of 3.6 ml of 0.05 *N* thiosulfate. For sweet potatoes the corresponding limits are 50% conversion and a titration of 5.4 ml. When these precautions are taken, the calculated number of units corresponds within experimental error to the observed results when flour is used as supplement (Table III). The correspondence between units calculated and units found for sweet potatoes is not valid for all the malts tested (Table IV). If any general inference is to be drawn concerning the lack of correspondence between found and calculated values, one may conclude that the found values tend to be higher for malts having low beta to alpha ratios (Table I). These findings are in accord with the supposition that there is present in sweet potato a system of glycosidases capable of degrading the lower dextrans and sugars formed by alpha-amylase action.

TABLE III

ADDITION OF SULFITE EXTRACT OF FLOUR AS SUPPLEMENTARY DIASTATIC SOURCE—  
APPARENT AMYLASE VALUES OBTAINED BY CONCOMITANT ACTION OF  
MALT AND SUPPLEMENT

Malt	Digestion mixture		Units (calculated)			Maltose equivalent apparent (calculated)	°L apparent	
	Malt	Supplement	Malt	Supplement	Total		Calculated	Found
Norwegian	<i>mg/5 ml</i> 0.625	<i>mg/5 ml</i> 0.173	520	109	629	1005	251	243
Ezond	0.625	0.173	441	109	550	881	220	222
	0.625	0.259	441	163	604	966	242	240
Atlas	1.250	0.345	342	217	559	448	112	109
	0.625	0.345	171	217	388	621	155	147
	0.625	0.528	171	326	497	795	199	198
	0.625	0.690	171	433	604	966	242	244
Commercial I	1.250	0.345	470	217	687	550	137	136
	0.625	0.345	235	217	452	720	180	172
	0.625	0.528	235	334	569	911	228	219
	0.625	0.690	235	436	671	1072	268	275
Commercial II	0.625	0.173	436	109	545	873	218	220
	0.625	0.259	436	164	600	960	240	241
Commercial III	1.250	0.345	315	217	532	426	106	108
	0.625	0.345	158	217	375	600	150	152
	0.625	0.528	158	334	492	788	197	188
	0.625	0.690	158	436	594	951	238	240
	0.625	0.862	158	544	702	1122	281	273
Commercial IV	1.250	0.345	402	217	619	496	124	122
	0.625	0.345	201	217	418	669	167	168
	0.625	0.528	201	334	535	856	214	208
	0.625	0.690	201	436	637	1018	254	247

### Alcoholic Fermentation

To obtain comparable results on the increased efficacy of fermentation due to the presence of supplement, the grain bill was calculated so that the weight of dry matter and the total amylase were identical in each case (Table V), the amylase being brought up to the level of that present in a 5% malt mixture of the most powerful malt used, *Kindred*. When the fermentation is allowed to proceed for 24 hours in the manner described, it can be seen (Table VI) that sweet potato (on an equi-amylolytic basis) is a more efficient supplement for alcohol production than is flour. Furthermore the three highest malts, whether beta-supplemented or not, gave the best alcohol yields. Again this is in accordance with the previous observations on the synergistic nature of the concomitant action of high alpha-amylase malt and sweet potato. As expected, there is no obvious relation between the Lintner values of

the malts and alcohol yields in the presence of supplements. In no case did the yield exceed that obtained with the "reference" malt, *Kindred*. It is of interest to note that the malt labeled "Commercial IV," possessing a very low beta to alpha ratio, performed most efficiently of all the malts used in the presence of supplement.

TABLE IV

ADDITION OF DRIED SWEET POTATO JUICE AS SUPPLEMENTARY DIASTATIC SOURCE—  
APPARENT AMYLASE VALUES OBTAINED BY CONCOMITANT ACTION OF  
MALT AND SUPPLEMENT

Malt	Digestion mixture		Units (calculated)			Maltose equivalent apparent (calculated)	°L apparent	
	Malt	Supplement	Malt	Supplement	Total		Calculated	Found
	<i>mg/5 ml</i>	<i>mg/5 ml</i>						
Norwegian	0.625	0.02	520	104	624	998	249	265
Ezond	0.625	0.02	441	104	545	872	218	217
	0.625	0.03	441	156	597	955	239	245
Atlas	1.250	0.04	342	208	550	440	110	109
	0.625	0.04	171	208	379	606	151	128
	0.625	0.06	171	312	483	772	193	202
	0.625	0.08	171	416	587	940	235	222
Commercial I	1.250	0.04	470	208	678	542	136	138
	0.625	0.04	235	208	443	709	177	177
	0.625	0.06	235	312	547	875	219	224
	0.625	0.08	235	416	651	1042	261	255
Commercial II	0.625	0.03	436	156	592	947	237	228
	0.625	0.05	436	260	696	1130	282	258
Commercial III	1.250	0.04	315	208	523	418	105	103
	0.625	0.04	158	208	366	585	146	145
	0.625	0.06	158	312	470	752	188	189
	0.625	0.08	158	416	574	918	230	236
Commercial IV	1.250	0.04	402	208	610	488	122	135
	0.625	0.04	201	208	409	654	164	172
	0.625	0.06	201	312	513	821	205	210
	0.625	0.08	201	416	617	988	247	271

TABLE V

USE OF SUPPLEMENT FOR FERMENTATION—SUPPLEMENTARY QUANTITIES  
REQUIRED FOR EQUIAMYLOLYTIC MASHES

Malt variety	Percent of grain bill due to		
	Malt	Malt + flour	Malt + sweet potato
Kindred	5.00	(5.00)	(5.00)
Norwegian	5.00	6.45	6.22
Ezond	5.00	7.45	7.07
Atlas	5.00	10.86	9.96
Commercial I	5.00	10.06	9.26
Commercial II	5.00	7.52	7.11
Commercial III	5.00	11.04	10.10
Commercial IV	5.00	10.48	9.62

TABLE VI  
USE OF SUPPLEMENTS FOR FERMENTATION—ALCOHOL YIELDS FOR 24 HOURS,  
PROOF GALLONS PER 100 POUNDS

Malt variety	Malt	Malt + flour	Malt + sweet potato
Kindred	9.5	(9.5)	(9.5)
Norwegian	8.1	8.8	9.3
Ezond	7.6	6.9	8.5
Atlas	6.4	7.1	8.1
Commercial I	6.8	—	8.4
Commercial II	7.4	8.3	8.5
Commercial III	6.4	—	8.9
Commercial IV	7.7	9.2	9.5

### Summary

A study has been made of the concomitant saccharifying action of various barley malts in the presence of sweet potatoes or flour, each as a supplementary source of beta-amylase. The limits of enzyme concentrations within which the rate of reaction is proportional to the enzyme concentration and within which valid comparison of this action can be made have been determined. For flour, this limit is that amount of enzyme which will cause not more than 30% starch conversion under the stated conditions and not more than 50% for sweet potato amylase. Within these limits, it has been found that the calculated activity of malt and flour mixtures is about the same as the experimentally determined activity, whereas the latter value tends to be higher than that calculated for mixtures of sweet potato and malts high in alpha-amylase activity. When mixtures of malts and supplement are used in fermentation tests in equiamyolytic amount, the subsequent yield of alcohol is greater for the sweet potato supplemented mashes. These results, which consistently demonstrate more extensive action in the presence of sweet potato, are consistent with the demonstration of appreciable concentrations of nonamyolytic enzymes therein concerned with sugar transformation.

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## ANNOUNCEMENT

**Bibliography and Review of the Scientific Literature on the Deterioration of Fats and Oils.** By B. F. Daubert, Quartermaster Food and Container Institute, Chicago, Illinois.

The Committee on Food Research, Office of the Quartermaster General, is making available a bibliography and review of the scientific and technological literature related to the deterioration of fats and oils, prepared by Dr. B. F. Daubert, Research Professor, University of Pittsburgh.

This material is printed on cards and the first 661 of a total 7000 were ready for mailing early in December. Only one set will be sent, upon request, to research organizations interested in this subject. The cards will be mailed in groups of a few hundred at a time as they come off the press.

Requests, on organization letterhead, should be addressed to George Gelman, Technical Director, Quartermaster Food and Container Institute, 1849 West Pershing Road, Chicago 9, Illinois.

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## THE RELATION OF ALPHA-AMYLASE AND SUSCEPTIBLE STARCH TO DIASTATIC ACTIVITY

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Many workers have interested themselves in the relationship existing between the amylolytic enzymes of wheat and their natural substrate, raw starch. Likewise, the value of the diastatic activity figure as an indication of the potential gassing power of a flour has been the subject of much investigation and discussion. The early workers recognized that "baking strength," generally measured in terms of loaf volume, depends largely on continued sugar formation in the dough and is therefore related to the diastatic activity.

The theoretical aspects of the subject were examined by Landis and Frey (1933, 1936), who concluded that no single diastatic or saccharogenic value will give more than an approximate picture of the fermentation potentialities of a flour. They concluded that since saccharogenesis is a logarithmic function, while fermentation is essentially a linear function of time, diastasis frequently becomes the limiting factor in normal fermentation.

As early as 1879 Brown and Heron, and in 1904 Maquenne, recognized that injury to starch granules resulted in greater rapidity of diastatic attack. Alsberg (1927) confirmed this and postulated two other factors influencing fermentation rate, namely, the relative number of uninjured granules and their susceptibility. In 1924 Rask and Alsberg, in reporting a viscosimetric study of wheat starches, recalled Whympers' (1909) observations that wheat starches vary in their resistance to diastatic action according to the quantity of gluten, and they expressed doubt as to whether the diastatic figure for a flour is necessarily an expression of enzymic content alone. Collatz (1922), as quoted by Rask and Alsberg (1924), found that the starch of strong flours appeared to be more easily hydrolyzed by diastatic enzymes than that of weak flours.

Hermano and Rask (1926) also stated that starches, either raw or gelatinized, vary in susceptibility to amylases, there being no connection between the relative susceptibility in the two conditions. Landis and Frey (1933), in discussing some terms used in cereal chemistry, outlined the two factors operating in autolytic saccharogenesis, enzyme activity, and starch susceptibility. After one hour, when the small amount of highly susceptible starch has been used up, the susceptibility of the native starch is the determining factor.

Andrews and Bailey (1934) stated that the diastatic activity in normal sound wheat is due primarily to beta-amylase and they concluded that diastatic activity is more a function of the starchy substrate than of amylase activity. Read and Haas (1936) expressed the opinion that the physical condition of the starch granules influences the quantity of maltose formed.

Sandstedt, Blish, Mecham, and Bode (1937) noted that the susceptibility of starch to amylase varies in different flours but that the raw uninjured starch in these flours has the same susceptibility to a given amylase preparation. Jones (1940) studied the production of mechanically damaged starch in milling and found that at least a portion of the differences in diastatic activity between flours is due to differences in the physical hardness of the endosperm; those flours from a hard wheat have, in general, a higher maltose figure than those from a soft wheat.

The "diastatic activity" or "maltose figure," expressing the relationship between the amylolytic enzymes and their starch substrate, has been and is widely used by control laboratories as an indication of the potential gas-forming capacity of a flour. The maltose as reported originates from several different sources. There is a certain small residual amount in the flour but the greater part is formed by the action of the amylolytic enzymes during the autolytic digestion. These enzymes, two or more in number, act on a starchy substrate which may be divided on a chemical basis into two fractions, i.e., amylose and amylopectin. On a physical basis the starch is made up of variable quantities of at least three fractions. There is, therefore, the possibility of considerable variation in the amount of maltose formed.

Some of the raw or native starch granules appear visually, with the aid of iodine and congo red staining, to be intact and others to be damaged by the milling process, while still another group is apparently undamaged but stains red as do the damaged granules. Evidence indicates that the number of granules capable of being stained red is related to the amount of maltose formed (Pulkki, 1938; Dadswell and Wragge, 1940). If a more satisfactory method were available for

measuring this portion of the starch, greater progress could be made in understanding the part it plays in maltose production. In this paper it is proposed to outline such a method and to demonstrate its use in studying the variations in maltose production in flour.

Important as the substrate is in determining the magnitude of maltose production, the amount of enzyme is likewise very important and an interpretation of maltose formation is incomplete without noting the effect of variation in the amount and type of amylase.

To accomplish the objects outlined above, quantities of beta- and alpha-amylases prepared from wheat were needed, as well as amylase-free flour. In addition to the usual diastatic activity value of the original flour, an indication of the amounts of alpha-amylase, of susceptible starch, and of maltose originally present in the flours was required. Such information could then be subjected to a statistical analysis in an effort to interpret the experimental findings.

### Materials and Methods

*Materials.* The flours studied were milled from six varieties of Australian white winter wheat grown in 1941 by the Department of Agriculture of Victoria at three places. The six varieties used, Regalia, Quadrant, Balmain, Dundee, Ghurka, and Pindar, were chosen because they represent a wide range in maltose and gas production.

*Preparation of Amylase-free Flour.* Five grams of flour mixed with sand and suspended in 25 ml of 0.075 *N* hydrochloric acid at 20°–22°C were mixed by rotating the flask every 15 minutes. Forty-five minutes after the addition of acid, 1.88 ml of 1 *N* sodium carbonate were added, followed by 7.62 ml of water. Finally, within two minutes of adding the sodium carbonate and water, 11.5 ml of buffer solution, the concentration of which is four times that recommended in the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935), were added at 30°C and digestion was carried out for one hour at this temperature, shaking every 15 minutes as in the usual autolytic digestion. Reducing sugars were then determined. Complete destruction of the amylases was shown by determining reducing sugars before and after digestion at 30°C.

In adding either beta- or alpha-amylase to the amylase-free flour the above procedure was followed except that only 4.62 ml of water (instead of 7.62 ml) were added, followed by 3 ml of water containing the quantity of enzyme which was desired. When enzyme was added, a correction had to be applied for its reducing value and all values obtained were corrected for the maltose originally present in the flour.

*Preparation of Enzymes.* The methods of extraction and precipita-



tion outlined below are based on those described by Sherman, Caldwell, and Doebbeling (1934) and later employed by Blish, Sandstedt, and Mecham (1937), and Kneen, Sandstedt, and Hollenbeck (1943). The differential inactivation of the two enzymes was carried out according to the technique first developed for malt amylases by Ohlsson (1930) and applied to those of wheat by Creighton and Naylor (1933).

*Beta-Amylase.* Ungerminated wheat of average quality was ground in a laboratory mill. Portions of 160 g were treated with 600 ml of 5% sodium chloride solution for 45 minutes with frequent stirring. The suspension was centrifuged and the insoluble residue extracted with a small amount of sodium chloride solution. The total volume of extract obtained was 660–670 ml.

Thirty-five grams of solid ammonium sulfate were dissolved in each 100 ml of clear extract. The resulting precipitate was separated by filtering, placed in a cellophane bag, and dialyzed for 2–3 days against running tap water with a low calcium content until free from sulfate. The contents of the bag were evaporated to dryness in a current of air at room temperature.

To destroy any alpha-amylase, each 10 g of the dry powdered enzyme preparation was mixed with 500 ml of water, cooled to 4°C, and enough 1 *N* hydrochloric acid added to bring it to pH 3.3. After 30 minutes at this low temperature the suspension was restored to pH 6 with 1 *N* sodium carbonate. The insoluble material was removed by filtration and the clear yellowish-brown solution evaporated to dryness in cellophane bags as before. The residue was finally dried in a vacuum at room temperature, finely ground, and sieved to give a uniform powder. The keeping quality of the product was excellent and it was judged to be free of alpha-amylase, since increasing concentrations, when added to a standard enzyme-free flour substrate, did not bring about any increase in dextrin formation.

*Alpha-Amylase.* Wheat of the same type as was used in the preparation of beta-amylase was germinated to a sprout length (plumule) of about a quarter of an inch. The germinated wheat was dried at room temperature in a current of air and ground in a laboratory mill. Extraction of the enzyme, precipitation with ammonium sulfate, and subsequent dialysis were carried out as for beta-amylase.

After addition of calcium acetate, according to the procedure suggested by Kneen, Sandstedt, and Hollenbeck (1943), in such an amount that 10 mg were present for each 5 ml of the contents of the dialyzing bags, the sulfate-free suspensions were maintained at a temperature of 75°C for 15 minutes to destroy the beta-amylase present. The suspension was then dialyzed to eliminate the calcium acetate, evaporated to dryness, and ground. The insoluble material

was not removed from this preparation before drying because the activity was of such a high order that its manipulation would not have been facilitated by further concentration.

Evidence that this alpha-amylase preparation was free from beta-amylase was obtained by use of a time-temperature inactivation curve of the type investigated by Blom, Bak, and Braae (1937), but using an amylase-free flour as substrate. A straight line was obtained, with a gradual slope, which indicated the absence of any beta-amylase.

Further confirmation of freedom from beta-amylase was obtained by examination of a curve relating the dextrinogenic and saccharogenic activity of the alpha-amylase preparation on raw starch after heating for varying periods. The regular shape of the curve indicated that beta-amylase was absent, since if it had been present there would have been initially a large decrease in maltose formation which was out of proportion to the decrease in dextrin production.

Kneen (1944, 1945) has reported that only small amounts of beta-amylase are present in a sorghum malt extract and it was found that the two methods described above were sufficiently sensitive to detect this.

*Analytical Methods Used.* The experimental results corresponding to the variables enumerated below are given in Table I. For each variable a symbol has been indicated, and this has been used in the tables, in the figures, and to some extent in the text.

*Maltose Originally Present (R).* Estimation of the maltose originally present was carried out using the same reagents as were needed for the determination of diastatic activity according to the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935). To avoid any amylase action, it was found necessary to add the buffer and sulfuric acid previously mixed together, followed immediately by the sodium tungstate.

*Susceptible Starch.* The term "susceptible starch" has been used in this paper to denote that portion of the starch granules which may be estimated directly by staining, or indirectly by the use of beta- or alpha-amylases. The methods were:

(1) The staining technique carried out by Dadswell and Wragge (1940) (S).

(2) The use of beta-amylase acting on amylase-free flour ( $M_\beta$ ). This is termed the "maltose formed by excess beta-amylase" for purposes of this discussion and may be defined as the maximum amount of maltose, in milligrams, which can be formed by allowing an excess of beta-amylase to act on 10 g of amylase-free flour in a buffered solution at 30°C for one hour. Details of manipulation are given under "preparation of amylase-free flour."

(3) The action of alpha-amylase on amylase-free flour ( $M_\alpha$ ). This is termed the "maltose formed by alpha-amylase" and it may be defined as the amount of reducing sugar, expressed as milligrams of maltose, produced by 160 mg of crude alpha-amylase when allowed to act on 10 g of amylase-free flour in a buffered solution at 30°C for one hour.

*Combined Action of Amylases and Susceptible Starch.* (1) Gross diastatic activity figure is the term used to include all the maltose formed during an autolytic digestion of flour and is considered to be derived from at least three sources. The main source is that supplied by the action of beta-amylase on the damaged or susceptible starch and this is influenced mainly by the amount of susceptible starch. The maltose formed thus is probably identical with the value designated above as maltose formed by excess beta-amylase and termed  $M_\beta$ .

The second source is that supplied by the action of alpha-amylase on susceptible starch, its magnitude being dependent on the amounts of alpha-amylase and susceptible starch.

A third and minor source of maltose derives from the action of beta-amylase on any material rendered susceptible to it by previous alpha-amylase action. If there are other amylases present they would contribute a fourth source of maltose.

By means of an amylolytic digestion carried out in steps, a better understanding was obtained of the relationship existing between alpha- and beta-amylase and between each amylase and the substrate. Amylase-free raw wheat starch was digested with an excess of beta-amylase at 30°C and washed free of soluble sugars. This material was subjected to a series of amylolytic digestions by allowing successive small equal amounts of either beta- or alpha-amylase to act at 30°C in buffered solutions. The amylase was destroyed and all soluble sugars were removed after each digestion. When the alpha-amylase digestions were interspersed with beta-amylase treatments, it was found that the amount of maltose due to alpha-amylase action was not significantly different from that obtained when no beta-amylase was used, but the amount of maltose due to beta-amylase action was increased as a result of a previous digestion with alpha-amylase, although successive treatments with beta-amylase alone gave practically no maltose. On the basis of this starch degradation, the second source of maltose in the gross diastatic activity figure is independent of the presence of beta-amylase and the third source, although due to beta-amylase, is dependent on some alpha-amylase activity having previously taken place.

For natural flours the gross diastatic activity figure ( $A_g$ ) may be defined as the reducing sugar, expressed as milligrams of maltose per

10 g of flour, which has been *formed during digestion* at 30°C for one hour under the conditions specified by the Blish and Sandstedt method (*Cereal Laboratory Methods*, 3rd ed., 1935). This value differs from the ordinary diastatic activity figure in that it is corrected for the reducing sugars present originally in the flour.

For artificial flours the gross diastatic activity figure ( $A'_g$ ) may be defined as for a natural flour, except that preparations of beta- and alpha-amylase were substituted in amylase-free flours for the amylases occurring in the natural flours. In these experiments 120 mg of crude beta-amylase and 140 mg of crude alpha-amylase per 10 g of flour were used.

(2) Net diastatic activity figure is the term used to designate that portion of the maltose which is formed during the gross diastatic activity estimation, and which owes its origin either directly or indirectly to the presence of amylases other than beta-amylase. It includes the second, third, and fourth sources of maltose, as noted above under the sources of maltose in the gross diastatic activity figure.

For natural flours the net diastatic activity ( $A_n$ ) may be defined as the difference between the gross diastatic activity ( $A_g$ ) and the maltose formed by excess beta-amylase ( $M_\beta$ ), and is expressed as milligrams of maltose per 10 g of flour.

For artificial flours the net diastatic activity figure ( $A'_n$ ) may be defined as the difference between  $A'_g$  and  $M_\beta$ , and is expressed as milligrams of maltose per 10 g of flour.

*Alpha-Amylase (D).* The alpha-amylase content of the flours was estimated by determination of the dextrin figure as carried out by the method of Kent-Jones and Amos (1940).

## Results and Discussion

The analytical data are presented in Table I. The samples are arranged in decreasing order with respect to their susceptible starch values as based on maltose formed by excess beta-amylase.

*Variety and Place Effects.* The effects of variety and place in relation to the combined action of susceptible starch and alpha-amylase, as well as in relation to each factor individually, are shown by the analysis of variance in Table II. The three measures of starch susceptibility ( $S$ ,  $M_\beta$ ,  $M_\alpha$ ) indicate that variety exerts a significant effect since the variety means differ significantly among themselves. Locality means, however, differ significantly only in the cases where susceptibility of the starch is measured as maltose formed by beta- or alpha-amylase. The limitations of the method of direct measurement of starch susceptibility ( $S$ ) may account for the fact that this does not show a similar relation to the variation between means of places.

TABLE I  
REDUCING SUGAR CONTENT, STARCH SUSCEPTIBILITY, DIASTATIC ACTIVITY, AND DEXTRIN VALUES  
FOR THE DIFFERENT WHEAT FLOURS

Variety	Place	Sample number	Reducing sugars in original flour as maltose	Susceptibility of substrate as measured by			Combined effect of susceptibility of substrate, alpha- and beta-amylase expressed as maltose						Amount of alpha-amylase in original flour as shown by dextrin figure
				Starch granules 20 $\mu$ diameter or over which stain with iodine-congo red	Maltose by excess beta-amylase <sup>1</sup>	Maltose by 160 mg crude alpha-amylase <sup>1</sup>	Gross diastatic activity <sup>2</sup>		Net diastatic activity				
							For natural flours	For artificial flours	For natural flours	For artificial flours			
R	S	M $\beta$	M $\alpha$	A $\beta$	A' $\beta$	A $\alpha$	A' $\alpha$	D					
	mg	%	mg	mg	mg	mg	mg	%					
Baldmin	30	19.4	141	219	188	558	47	417	4.5				
Dundee	34	13.2	123	224	276	608	153	485	12.5				
Baldmin	36	16.2	104	224	178	543	74	439	6.0				
Dundee	44	15.2	103	188	182	532	79	429	4.5				
Baldmin	26	18.5	96	196	166	503	70	407	5.0				
Dundee	34	17.0	76	157	141	417	65	341	4.5				
Regalia	38	10.2	67	150	135	359	68	292	6.5				
Quadrat	23	8.0	67	150	92	315	25	248	4.0				
Ghurka	26	9.4	66	139	105	343	39	277	5.0				
Regalia	38	12.8	61	164	143	342	82	281	8.5				
Pindar	43	11.3	60	122	104	329	44	269	5.5				
Regalia	30	12.8	57	134	102	333	45	276	6.0				
Ghurka	47	8.8	57	134	103	359	46	302	5.0				
Pindar	30	8.5	56	144	92	296	36	240	5.0				
Quadrat	40	10.4	51	143	94	337	43	286	5.0				
Ghurka	26	8.4	43	112	84	276	41	233	5.0				
Quadrat	24	8.9	36	105	68	249	32	213	5.0				
Pindar	28	7.4	36	102	69	242	33	206	5.0				

<sup>1</sup> Determinations made on amylase-free flour.

<sup>2</sup> Corrected for reducing sugars (R) present in original flour.

The gross diastatic activity figures of both natural and artificial flours ( $A_g$ ,  $A'_g$ ) are related to variety and place. This is to be expected since one of the measures of starch susceptibility ( $M_p$ ) is considered to be a part of the gross diastatic activity figure. Consideration of the net diastatic activity ( $A_n$ ,  $A'_n$ ), where the variation in alpha-amylase should be more apparent, shows that variety means differ significantly for both natural and artificial flours, but only artificial flours containing uniform amounts of alpha-amylase show a significant variation between means of places. The same effect is somewhat apparent in the variations between means of places for the gross diastatic activity, since the significance of the difference between means is of a higher order for the artificial flours than for the natural flours.

No significant difference exists between means of varieties or places for alpha-amylase (D) and, therefore, the amount of alpha-amylase is not a feature of locality or of variety.

*Starch Susceptibility.* The least satisfactory of the three methods used for measurement of starch susceptibility is that involving the staining technique. Results obtained by this method were found to vary slightly with different individuals. The data enumerated in Table I were obtained by one individual. In any case it is not a method which lends itself to a high degree of accuracy and it is very tedious. The results obtained are, however, of interest to use in comparison with the data obtained by other methods.

In employing amylases to investigate the susceptibility of starch, they may be used singly or in combination. In this investigation they were used singly, since a combination simulates the more complex system of a natural flour and the results obtained would, therefore, be comparable to what has been defined above as the gross diastatic activity.

It is generally conceded that all wheat flours contain an abundance of beta-amylase and varying amounts of alpha-amylase, with the possibility of an additional raw starch-splitting amylase. In the autolytic digestion of a flour, the beta-amylase has opportunity to attack any starch fraction susceptible to it as well as dextrins formed by the alpha-amylase present. Many of the flours studied show a low alpha-amylase content and in such a case the main activity of the beta-amylase is to attack the starch which is susceptible to its action. This can be investigated by rendering a flour amylase-free, adding beta-amylase and noting the maltose formed during digestion for one hour at 30°C. When sufficient beta-amylase was added to any of the 18 samples of flour, a limiting amount of maltose was obtained which could not be increased by longer digestion or by the addition of more of the enzyme. In actual practice the value obtained must, of course,

TABLE II  
ANALYSIS OF VARIANCE FOR THE EFFECT OF WHEAT VARIETY AND LOCATION OF GROWTH ON REDUCING SUGAR CONTENT,  
VARIOUS MEASURES OF STARCH SUSCEPTIBILITY AND DIASTATIC ACTIVITY, AND ON  $\alpha$ -AMYLASE  
CONTENT—MEAN SQUARES

Source of variation	Degrees of freedom	Susceptibility of substrate as measured by			Combined effect of susceptibility of substrate, alpha-, and beta-amylase expressed as maltose				Amount of alpha-amylase in original flour as shown by dextrin figure
		Starch granules 20 $\mu$ diameter or over which stain with iodine--congo red	Maltose by excess beta-amylase 1	Maltose by 160 mg crude alpha-amylase 1	Gross diastatic activity		Net diastatic activity		
					For natural flours	For artificial flours	For natural flours	For artificial flours	
		S	M $\beta$	M $\alpha$	A $_g$	A' $_g$	A $_n$	A' $_n$	D
Between means of varieties	5	43.83**	2,294.62**	4,108.7**	7,174.4**	37,107**	1,818.62**	21,073	3.58
Between means of places	2	1.59	1,291.55**	2,210.1**	2,886.0*	10,837**	373.55	5,311**	2.05
Remainder	10	2.55	81.29	119.3	631.6	1,071	510.29	768	4.41

<sup>1</sup> Determinations made on amylase-free flour.

\* Significant.

\*\* Highly significant.

be corrected for the maltose originally present in the flour so as to obtain the amount formed by the beta-amylase. The values for these samples ranged from 36 to 141 mg of maltose per 10 g of flour.

Fair agreement between the beta-amylase method and the staining technique for the measurement of susceptible starch is indicated by the over-all correlation of 0.798 which is highly significant (see Table III). A further series of 12 samples composed of three varieties grown at four places in 1939 gave an over-all correlation of 0.763 for the same pair of factors.<sup>1</sup> The relationship of these two estimates of susceptible starch is such that, for a 10 mg increase in maltose, the percentage of starch granules of 20 $\mu$  diameter or over which stain red increases by 1.03%.

TABLE III

CORRELATION COEFFICIENTS BETWEEN DIFFERENT MEASURES OF STARCH SUSCEPTIBILITY, DIASTATIC ACTIVITY AND DEXTRIN FIGURE

Variables correlated	Correlation coefficient
Maltose by excess $\beta$ -amylase and gross diastatic activity by crude $\alpha$ -amylase	$r_{M\beta M\alpha}$ 0.944**
Maltose by excess $\beta$ -amylase and injured starch granules 20 $\mu$ diameter or over	$r_{M\beta S}$ 0.789**
Maltose by crude $\alpha$ -amylase and starch granules 20 $\mu$ diameter or over which stain with iodine-congo red	$r_{M\beta S}$ 0.782**
Maltose by excess $\beta$ -amylase and gross diastatic activity for natural flours	$r_{M\beta A_g}$ 0.897**
Maltose by excess $\beta$ -amylase and gross diastatic activity for artificial flours	$r_{M\beta A'_g}$ 0.958**
Maltose by excess $\beta$ -amylase and net diastatic activity for natural flours	$r_{M\beta A_n}$ 0.609**
Maltose by excess $\beta$ -amylase and net diastatic activity for artificial flours	$r_{M\beta A'_n}$ 0.926**
Maltose by excess $\beta$ -amylase and dextrin figure	$r_{M\beta D}$ 0.301
Dextrin figure and gross diastatic activity for natural flours	$r_{DA_g}$ 0.644**
Dextrin figure and net diastatic activity for natural flours	$r_{DA_n}$ 0.853**

.16 degrees of freedom.

\*\* Highly significant.

The third method for estimation of susceptible starch is based on the action of alpha-amylase alone on amylase-free samples of the flours studied. While this is a condition probably never present in natural flours it is of interest because of the manner in which the flours reacted. In Table I are given the responses to a uniform amount of alpha-amylase of 18 samples rendered amylase-free in the manner described in the experimental section. These values are graphically

<sup>1</sup> Unpublished data. Dadswell, Wragge, and Gardner, 1942.



represented in Figure 1 and show the close relationship between the responses of a given flour to the two enzymes. The quantity of maltose formed by the alpha-amylase is, of course, a function of the amount of enzyme used as well as the time of digestion, which in this case was one hour at 30°C. It may be noted here that the time-maltose relationship of each of the amylases was found to be of the nature indicated in

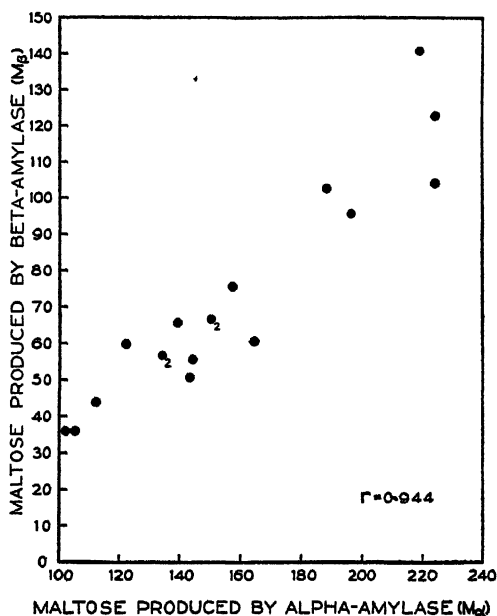


Fig. 1. Scatter diagram showing relation between maltose produced by a high level of alpha-amylase and by excess beta-amylase acting on amylase-free flours.

Figure 2. The curves illustrate the typical responses of three amylase-free flours to the two enzymes and show one of the great differences between the enzymes. The level of alpha-amylase chosen was high, so high that it is equivalent to a dextrin figure of about 30%, a value very much greater than is likely to be found in the case of a commercial flour sample. Even at this high level, the amount of alpha-amylase was insufficient to react with all the susceptible starch available in the time allowed. This constitutes one of the main differences between the two amylase methods for evaluating the susceptibility of starch.

Maltose due to alpha-amylase ( $M_\alpha$ ) is closely related to the amount of susceptible starch determined by the staining technique, as shown by their correlation coefficient of 0.782. The correlation of 0.944, indicating the relationship between maltose due to beta-amylase ( $M_\beta$ ) and that due to alpha-amylase ( $M_\alpha$ ), is expected on account of

the close relationship of each with susceptible starch as determined by the staining method (S).

Some value such as  $M_s$  could be generally used as a measure of susceptible starch but the use of beta-amylase is more convenient and practical at present. The measurement of susceptible starch can, therefore, be most conveniently carried out by the use of beta-amylase acting on amylase-free flour.

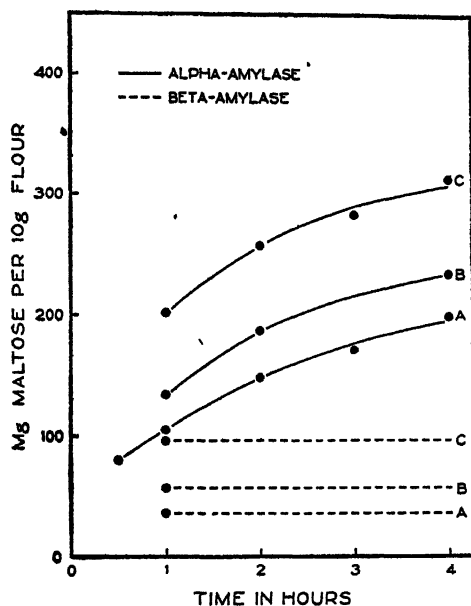


Fig. 2. Curves showing maltose production in relation to time for three amylase-free flours (A, B, and C), treated with alpha- and with beta-amylases

*Combined Action of Amylases and Susceptible Starch.* The correlation between susceptible starch ( $M_s$ ) and gross diastatic activity ( $A_g$ ) has the highly significant value of 0.897. If starch susceptibility were measured by the staining technique (S) the correlation was also highly significant (0.694), this value being slightly reduced (0.683) if calculated on the basis of the ordinary diastatic activity, i.e., not corrected for the reducing sugars originally present in the flour. When calculated on the latter basis, a previous series of 24 samples (Dadswell and Wragge, 1940) showed a correlation of 0.954. A third series, representing four varieties grown at four places over a period of two years, gave a correlation of 0.750.<sup>2</sup>

For the artificial flours the correlation between susceptible starch ( $M_s$ ) and gross diastatic activity ( $A'_g$ ) was 0.958. The gross diastatic

<sup>2</sup> Unpublished data. Dadswell, Wragge, and Gardner, 1942.

activity figures for a series of artificial flours where standardized amounts of amylases were used constitute another estimate of susceptible starch; therefore, the correlation of 0.958 represents the relation between the data obtained by two methods of measuring susceptible starch, and as such its magnitude compares favorably with  $r_{M_{\beta}M_{\alpha}} = 0.944$ .

The values obtained for maltose due to beta-amylase ( $M_{\beta}$ ) were found, in the case of each of the 18 flours, to be less than their gross diastatic activity figures (see Table I). That there is such a positive difference in favor of the gross diastatic activity figure lends support to the idea that all these flours contain one or more amylolytic enzymes, other than beta-amylase, which are capable either of forming maltose directly, or else of forming dextrans which can be split to form maltose. In one old sample of flour the gross diastatic activity figure and the maltose due to beta-amylase were nearly the same, giving values of 147 and 136 mg, a difference of 11 mg in favor of the gross diastatic activity figure. It has been noted that, as flours age, it is the net diastatic activity figure which decreases, not the maltose due to beta-amylase.

Flours having a low alpha-amylase content were found to form only small additional amounts of maltose if the original autolytic digestion period of one hour were prolonged by another hour. This indication that the beta-amylase normally present is able, within one hour at 30°C, to convert to maltose all the susceptible starch, together with the fact that addition of beta-amylase in a diastatic activity estimation does not increase the maltose value obtained, suggests that the formation of maltose during a diastatic activity determination in excess of that which beta-amylase can produce when present alone is due, directly or indirectly, to other amylases present in the original flour.

The net diastatic activity figures ( $A_n, A'_n$ ) are less closely correlated with susceptible starch than are the corresponding gross diastatic activity figures, although the difference between  $r_{M_{\beta}A'_n}$  and  $r_{M_{\beta}A_n}$  is not significant. The variability of alpha-amylase in the natural flours is one of the main reasons for  $r_{M_{\beta}A'_n}$  being greater than  $r_{M_{\beta}A_n}$ . The dextrin figure was significantly correlated with the gross diastatic activity and the net diastatic activity.

The multiple correlation coefficient for the natural flours representing the relation between the factors susceptible starch, alpha-amylase, and gross diastatic activity is 0.979. The correlation between the first two factors and the net diastatic activity is 0.930 which compares favorably with  $r_{M_{\beta}A'_n} = 0.926$ , where the variability of alpha-amylase is not a factor to be considered.

*Estimation of Maltose Formation.* The standard deviation of the gross diastatic activity figure for the original 18 samples was 53.2 mg per 10 g of flour (Table IV). This was reduced to 24.3 mg by taking into account the susceptibility of the starch to beta-amylase, and was further diminished by consideration of the amount of alpha-amylase to give a standard error of estimate of 11.6 mg. In terms of variance this means that about 4% of the variance of the gross diastatic activity figure is still unaccounted for.

In the case of the artificial flours, the standard deviation of the gross diastatic activity figure is 113, which is reduced to a standard error of estimate of 33. This leaves about 8% of the variance still unaccounted for and compares favorably with the amount of variation unaccounted for in the natural flour.

TABLE IV  
ORIGINAL AND RESIDUAL VARIANCES OF DIASTATIC ACTIVITY<sup>1</sup>

Original variance for	Residual variance after correction by regression on			Percentage, residual variance of original after correction by			Percentage of original variance accounted for by		
	$M\beta$	$D$	$M\beta D$	$M\beta$	$D$	$M\beta D$	$M\beta$	$D$	$M\beta D$
$A_g$ 2830.2	553.0	1656.4	117.6	19.5	58.5	4.2	80.5	41.5	95.8
$A_n$ 882.1	554.9	240.3	119.2	62.9	27.2	13.5	37.1	72.8	86.5
$A'_g$ 12814.2	1053.8	—	—	8.2	—	—	91.8	—	—
$A'_n$ 7259.0	1034.6	—	—	14.2	—	—	85.8	—	—

Percentage of residual  $A_n D = 240.3$  accounted for by  $M\beta \beta$  50.4, unaccounted for by  $\beta$  — 49.6%.  
Percentage of  $A'_n$  accounted for by  $M\beta \beta$  85.7, unaccounted for by  $M\beta \beta$  14.3%.

<sup>1</sup> Key to symbols:

$A_g$  = Gross diastatic activity for natural flours.

$A'_g$  = Gross diastatic activity for artificial flours.

$A_n$  = Net diastatic activity for natural flours.

$A'_n$  = Net diastatic activity for artificial flours.

$D$  = Amount of alpha-amylase in original flour as shown by dextrin figure.

$M\beta$  = Maltose by excess beta-amylase.

The dependence of the gross diastatic activity figure on the susceptible starch becomes less strongly marked when alpha-amylase is included as a factor, the net regression coefficient for susceptible starch being reduced from 1.610 to 1.388,<sup>3</sup> but this is more than compensated for by the increase in the accuracy of the estimate brought about by introducing the second factor.

The standard deviation of the net diastatic activity figure for the original flours has been lowered from 29.7 to give a standard error of estimate of 11.6 mg per 10 g of flour by inclusion of the effects of sus-

<sup>3</sup> See equations, Figure 3.

ceptible starch and alpha-amylase. This leaves 14% of the variance unaccounted for.

The artificial flours have a standard deviation of 85 for the net diastatic activity figure which is reduced to a standard error of estimate of 33 by consideration of the effect of susceptible starch. The variance unaccounted for is 14% which is the same as for the natural flours. Such a comparison is open to question, since in one case the variance unaccounted for is based on conditions of uniform alpha-amylase

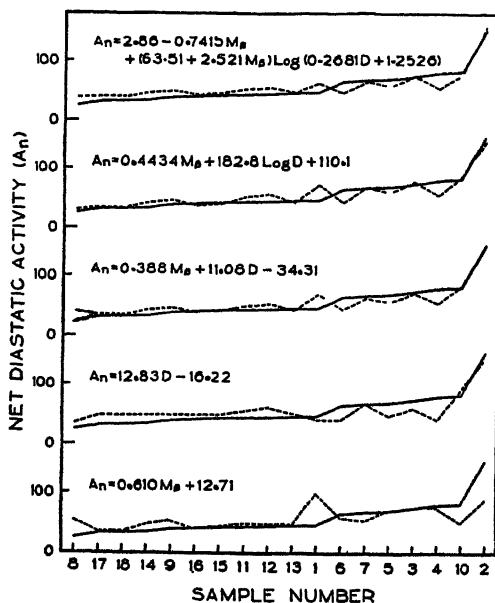


Fig. 3. Actual (—) and estimated (---) values of net diastatic activity.

content, while in the other case it is based on varying alpha-amylase content. A comparison can be made, however, after the variance due to alpha-amylase has been eliminated by calculation. On that basis the variance unaccounted for by susceptible starch is 7.1% in the case of the natural flours as compared with 8.2% in the case of the artificial flours, and 50% for the net diastatic activity of the natural flours as compared with 14% for artificial flours. That the variance unaccounted for is so much higher for the natural flours than for the artificial flours indicates that either the experimental error is much higher for natural flours or there is a possibility of some unrecognized variant being present in the natural samples.

Comparisons of the experimentally determined values for the net diastatic activity figure with those calculated from the susceptible

starch or the alpha-amylase content or from both factors have been made in Figure 3. Use of both factors together gives the best estimate of the net diastatic activity, but, as would be expected from the previous discussion, susceptible starch alone gives a better estimate than alpha-amylase, except in six cases. The curve obtained from the equation  $A_n = 0.4434M_\beta + 182.8 \text{ Log.D} - 110.1$  is also shown in Figure 3, and differs little from that based on the simpler relationship between susceptible starch and alpha-amylase.

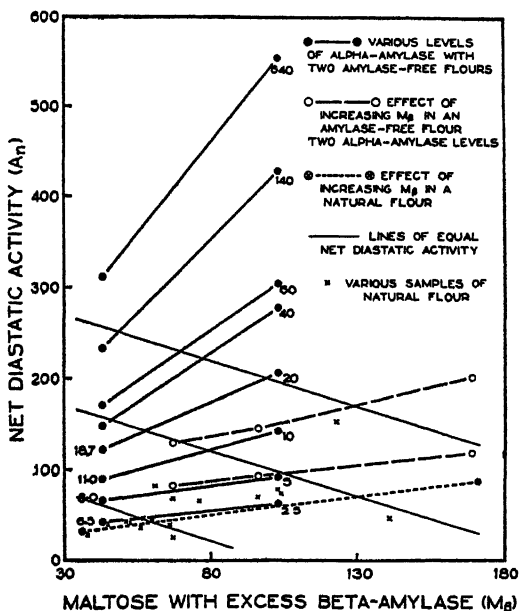


Fig. 4. The relationship between maltose produced by excess beta-amylase and net diastatic activity at various levels of alpha-amylase in the presence of excess beta-amylase. (The figures beside the curves on the right-hand side represent the units of alpha-amylase used per 10 g of flour; those on the left-hand side indicate the equivalent % dextrin.)

The 18 natural flours studied present a limited range of both alpha-amylase content and susceptible starch and, consequently, a prediction of the net diastatic activity for other combinations of the two factors is difficult. By using the data obtained from the treatment of amylase-free flour with an excess of beta-amylase in the presence of varying levels of alpha-amylase, it is possible to extend the information obtainable and so to predict the net diastatic activity of flours having a wide range of alpha-amylase and susceptible starch values.

It was observed (Figure 4) that the net diastatic activity figures of the artificial flours having a susceptible starch value of 43 are a logarithmic function of the amount of alpha-amylase used and of the percentage of dextrin, the equation being  $A_n = 171.9 \text{ Log}(0.2681D$

+ 1.2526) - 29.025. The increase in the value of the net diastatic activity over the range of susceptible starch from 43 to 103 is given by the expression  $[151.24 \text{ Log}(0.2681D + 1.2526) - 44.49] \frac{M - 43}{60}$ .

These two equations together give the value of the net diastatic activity figure corresponding to any value of susceptible starch between 43 and 103, and varying levels of alpha-amylase,

$$A_n = 2.860 - 0.7415M_\beta + (63.51 + 2.521M_\beta) \text{Log}(0.2681D + 1.2526).$$

The values calculated according to this equation for the 18 samples are shown in Figure 3, and compare favorably with those obtained from the other equations.

Dextrin values as given in Figure 4 for the artificial flours have been corrected so as to make them comparable with those obtained for natural flours. The relationship between the two series was found to be  $D = 3.002 \times \% \text{ dextrin for artificial flours} - 2.65$ . The cause of this discrepancy is not at present understood, but it is considered that the explanation may lie in the effect of the acid on the starch during the preparation of the artificial flour as a substrate, in a difference in the alpha-amylase in the preparation used from that as it exists in the natural flour, as it appears to be less active when isolated and allowed to react with amylase-free flour in the presence of excess beta-amylase, or in the possible presence of an activator in the natural flour which is absent from the artificial flour.

One point to be elucidated is the variable response, in terms of maltose production, of ordinary commercial flours with the same diastatic activity figure to the addition of equal amounts of alpha-amylase. Examination of Figure 4 may assist in clarifying this problem. The solid radiating lines, marked as being equivalent to certain levels of dextrin formation, were determined experimentally by subjecting amylase-free flours with different susceptible starch values to a given addition of alpha-amylase in the presence of an excess of beta-amylase. The dextrin values given have been corrected as indicated earlier so as to be comparable with those obtained for natural flours. Each line shows the effect of holding the alpha-amylase at a constant level while varying the value of susceptible starch, with its resultant effect on the net diastatic activity. Each of the three parallel lines running between the two axes represents a series of possible flours having the same gross diastatic activity figures, i.e., the values of the two axes add up to the same amount along a given line, for instance,  $M_\beta = 150$  plus  $A_n = 50$  equals a gross diastatic activity

of 200, or  $M_\beta = 75$  plus  $A_n = 125$  equals a gross diastatic activity of 200. The dot and dash lines represent the effect of varying the susceptible starch while keeping the alpha-amylase content the same. This was accomplished by grinding a flour very slightly in a ball mill and sampling it at intervals. In this case the amylases naturally present were not destroyed, nor were they augmented by any additions but were allowed to act in the usual autolytic digestion. These curves confirm the solid radiating curves which were made up by observing the behavior of amylase-free flour treated with preparations of alpha- and beta-amylases.

The effect of adding a given amount of alpha-amylase to flours with different susceptible starch values is dependent on whether  $M_\beta$  is large or small. If large, the enzyme is very effective in raising the net diastatic activity figure and hence the gross diastatic activity figure, but if  $M_\beta$  is small, an increase in alpha-amylase increases the net diastatic activity value relatively little. For instance, if  $M_\beta = 125$  and  $A_n = 49$ , with a resultant gross diastatic activity of 174, an increase in alpha-amylase equivalent to 6% dextrin will increase  $A_n$  to 135, that is by 86 mg of maltose, resulting in a gross diastatic activity of 260. On the other hand, if  $M_\beta = 50$  and  $A_n = 35$ , with a resultant gross diastatic activity of 85, an increase of the same magnitude in alpha-amylase will result in  $A_n$  being increased to 79, a net increase of only 44 mg of maltose with a gross diastatic activity of 129.

The variable response of commercial flours to addition of alpha-amylase is therefore capable of a simple explanation in that it depends to a considerable extent on the level of susceptible starch in the flour.

### Summary

Flours prepared from six varieties of white winter wheat, each grown at three places in Victoria, Australia, were studied with reference to their susceptible starch, alpha-amylase, and diastatic activity.

A corresponding series of artificial flours, with the same susceptible starch content as the natural flours but having a uniform amylase content, was also studied with reference to diastatic activity.

Susceptible starch was found to be related to variety and place of growth, but alpha-amylase bore no relation to either. In natural flours, containing variable levels of alpha-amylase and susceptible starch, the gross and net diastatic activity figures mainly reflected the effect of variety. In artificial flours the gross and net diastatic activity figures were both significantly related to place and variety effects.

The gross and net diastatic activity figures were significantly correlated with the susceptible starch and alpha-amylase contents of the natural flours, and with the susceptible starch of the artificial flours.



In the gross diastatic activity figures of the natural flours about 80% of the total variance is accounted for by the variability of the susceptible starch and an additional 16% by the alpha-amylase, leaving only 4% of the original variance still unaccounted for.

In the gross diastatic activity figures of the artificial flours about 92% of the total variance is accounted for by the variation in susceptible starch, which is similar to that accounted for by the same factor in the natural flours (96%) when calculated on the basis of uniform alpha-amylase content.

In the net diastatic activity figures of the natural flours about 37% of the variance is accounted for by the variability of the susceptible starch and about 73% by the alpha-amylase. When the susceptible starch and alpha-amylase are used together to predict diastatic activity the total variance accounted for is about 86%.

In the net diastatic activity figures of the artificial flours about 86% of the variance is accounted for by variation in susceptible starch. For the natural flours the variance for net diastatic activity after correction for the dextrin figure is 240.27 and this is reduced to 119.17 after taking into account the effect of variation in susceptible starch. The portion of 240.27 accounted for here is 121.10 or only 50%. This discrepancy suggests that some factor other than susceptible starch and alpha-amylase may be necessary to explain the variability of the net diastatic activity.

A better understanding of the variable response to alpha-amylase, in terms of maltose production in natural flours having the same gross diastatic activity figure, has been attained by reference to their susceptible starch content; flours having a high susceptible starch content giving a greater maltose production for a given amount of alpha-amylase than those having a low amount of susceptible starch.

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# A STUDY OF THE BEHAVIOR OF NONVIALE DRY YEAST IN BREAD DOUGH

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The general utilization of dry nonviable yeast as a food presents more of a problem than does its production. Brewers' by-product yeast and primary grown yeasts have been dried and made available to the pharmaceutical trade for special dietary uses for many years. In recent years, dehydration equipment and processes have come into more common use, and food and feed shortages, along with the pressure of wartime requirements, have increased the market demand for dry yeast.<sup>2</sup> The brewing industry has responded to this demand by increasing its yeast recovery to a potential of approximately 5 to 10 million pounds of dry yeast yearly with from 15 to 20 drying plants operating in breweries and yeast collecting centers. This represents only a portion of the ultimate potential, variously estimated at from 20 to 30 million pounds yearly. Primary grown yeast production has been perfected to give high yields, and in areas where fermentable carbohydrates are plentiful this could be made a source of high-quality protein and vitamin-rich food material.

Dry brewers' yeast has found an outlet in animal feed mixes, and, after debittering, has been used for direct human consumption as a special dietary supplement. Many food processors have found dry yeast useful in limited amounts in supplementing the vitamin and protein content and flavor qualities of their products. A more extensive use of dry yeast as a food will require special means of preparing or incorporating the yeast because the palatability of dried yeast does not make it generally acceptable.

Yeast is a natural component of yeast-leavened bread, and it would appear that additional yeast in the nonviable form would be a logical addition for enhancing its nutritional value. Such a procedure would afford a means for the ultimate consumption of this highly nutritious material. Incorporating dry yeast in bread was experimented with in Europe during the first World War and it was concluded that only limited amounts could be used to make a more nutritious yet palatable product. Schülein (1935) found that additions of 3% dry irradiated yeast to bread definitely improved its nutritional value.

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<sup>2</sup> The term dry yeast as used throughout this paper refers to nonviable dry yeast unless otherwise specified.

Schwarz, Laufer, Laufer, and Brenner (1942) found that additions of 2.5% and 5.0% (flour basis) of dry brewers' yeast to white bread made significant contributions to the vitamin B-complex content of the resulting loaf. Light and Frey (1943) found that the addition of 5% dry yeast, containing approximately 50% protein, made a very definite improvement in the nutritional value of bread, as measured by animal growth response tests, in comparison with growth response in a control, and with bread from a 6% nonfat milk solids formula. The growth response was proportional to the lysine increase from the respective additions of the dry yeast and the nonfat milk solids. Because white flour protein is somewhat deficient in this essential amino acid, dry yeast, which has a good proportion of lysine in its protein, becomes a logical supplement for improving the deficiency and establishing a better nutritional balance in the white bread.

Recently, McCollum (1945) has suggested the use of dry brewers' yeast and other protein-rich and vitamin-rich natural products as sources for "natural" enrichment. He indicated that bread supplemented with these materials gave loaves of good palatability, but which did not score high by conventional scoring methods. He suggested that if such materials were used, the consumer might have to modify his ideas of what constituted good quality bread.

Schwarz and co-workers found that the loaf score decreased as the amount of dry yeast was increased, and the addition of 2½% dry yeast produced a loaf scoring slightly below normal.

It is generally known by those who have included dry yeast in the dough batch that a pronounced dough softening effect is likely to be encountered when the dough is being mixed. The dough condition may improve to some extent during fermentation, but the deterioration persists throughout the breadmaking process, resulting in inferior bread.

Subsequent to the work of Schwarz *et al.* (1942) in this laboratory, it was found that commercial dry yeasts vary considerably in the degree to which they affect the physical characteristics of the dough. It appears that the sample of dry yeast with which they experimented might have been somewhat better than the average commercial dry yeast in this respect. An investigation of this variable factor in dry yeast forms the basis of this report.

### Materials and Methods

A number of dry yeast samples were obtained from widely scattered sources and subjected to comparative tests. A brief description of the yeasts is given in Table I. In some cases, the method of processing these yeasts was known, but no attempt was made to correlate the

processing treatments and drying methods with the experimental findings.

Because the deleterious effects of nonviable dry yeast in a bread dough are evident in the finished loaf, as well as in the mixing and handling of the dough, a baking test was used to evaluate the bread-making properties of the dry yeast samples.

TABLE I  
BREAD SCORES WHEN THE FORMULA INCLUDES 3% (FLOUR BASIS) OF VARIOUS DRIED NONVIALE YEASTS AS RECEIVED AND AFTER DIFFERENT TREATMENTS

Dried yeast sample		Mg iodine reduced by water extract from 1 g dried yeast (as received)	Bread score <sup>1</sup>		
Identification No.	Descriptive origin of yeast		Yeast used as received	Yeast used after treating with sodium chlorite, 1 mg/g yeast	Used after boiling 1 hour, then treating with sodium chlorite, 1 mg/g yeast
Control	—	—	100	—	—
L	Lager yeast, debittered	1.1	95	—	—
M	Primary grown nutritional yeast	2.4	92	—	—
I	Lager yeast, debittered	3.5	85	93	—
H	Ale yeast, debittered	4.3	84	94	—
D	Ale yeast, not debittered	4.3	84	94	—
G	Ale yeast, debittered	4.3	82	94	—
K	Ale yeast, not debittered	5.0	82	94	—
B	Lager yeast, not debittered	4.3	80	84	94
F	Primary grown nutritional yeast	5.3	80	88	94
N	Compressed bakers' yeast, laboratory dried <sup>2</sup>	5.3	76	88	92
O	Comm'l bakers' viable dry yeast <sup>2</sup>	3.7	76	88	94
J	Lager yeast, not debittered	4.7	75	84	94
A	Lager yeast, debittered	5.6	75	80	90
E	Lager yeast, debittered	5.9	75	86	93
C	Lager yeast, not debittered	5.6	50	40	92
15 mg % glutathione (GSH)			75	98 <sup>3</sup>	

<sup>1</sup> A score of 100 was assigned to the control in each day's bake. Scores of 95 and over: excellent; 90-94: good; 85-89: fair, acceptable; 80-84: poor; below 80: very poor.

<sup>2</sup> Suspension of the dried bakers' yeast pasteurized at 60°C for 15 minutes to kill viable yeast before use.

<sup>3</sup> 15 mg glutathione (GSH) in solution was satisfactorily oxidized by pretreatment with 4 mg sodium chlorite.

The baking test was based on the use of 400 g bakers' patent flour mixed in a C-10 Hobart-McDuffy type mixer. Other ingredients were water (62% flour absorption, 15% moisture basis), compressed yeast (3%), salt (2%), hydrogenated shortening (3%), and sugar (5%). Dry yeast was used in amounts of 3% and 5% (flour basis) and additional water was calculated at the rate of approximately 50% of the weight of the added dry yeast. A portion of the formula water was

used to incorporate the dry yeast in a slurry before adding it to the mixer with the other ingredients. The normal mixing time for the control was three minutes at low speed and two minutes at medium speed. When dry yeast was added, the mixing time at medium speed was reduced according to the necessity imposed by the dough-softening effect of the dry yeast under test; most of the samples required a reduction in mixing time of 15 to 30%.

Doughs were fermented at 27°–29°C for 105 minutes to the first punch, 45 minutes to the second punch, and 25 minutes to the pan. From each mix, two 10-ounce doughs were panned and proofed at 35°C for approximately 55 minutes. A constant height was used as the criterion for correct proof time unless the dough did not reach normal height in 60 minutes. In this case, the dough was placed in the oven regardless of height. The loaves were baked for 35 to 40 minutes at 230°C.

Sponge dough tests were also made to confirm the reliability of the indications from the straight dough procedure. Bread scoring was based principally on the factors of loaf volume and texture, grain, and color of the crumb because these were the characteristics most directly associated with the factors under study in these experiments.

### Experimental Results and Discussion

Preliminary studies with dry yeasts indicated that their inclusion in the bread formula had little effect on gas production. Also, the factor or factors in the dry yeast that caused the destructive effect on the physical character of the dough were found to be in the water-soluble extract of the dry yeast. When dry yeast made up into a slurry was centrifuged before incorporating in the mixer, and only the supernatant liquid used, the deleterious effect on the dough was equal to that of the entire yeast. When the yeast slurry sediment was washed and incorporated in the dough, only a slight harmful effect was observed.

Jørgensen (1936), Ford and Maiden (1938), and Swanson and Andrews (1945) have shown, with mixograms, a softening effect of the water extract of boiled yeast on bread dough similar to the action of glutathione in a dough. It was our experience that dry yeast, because of certain water-soluble factors, behaves in a dough similar to glutathione (GSH) or other reducing substances such as sulfites. When 15 mg glutathione per 100 g flour (15 mg percent) was included in the bread formula, the dough-handling properties and loaf score were similar to those obtained when 3% of the average dry yeast was used.

Figure 1 shows the effect on loaf characteristics of adding 3% (flour basis) of the various dried yeasts to the bread formula. Two

controls are used for reference. Loaves marked "Bl" represent the blank or control to which no dry yeast additions were made. The loaf marked "Gl" represents the loaf deterioration caused by the

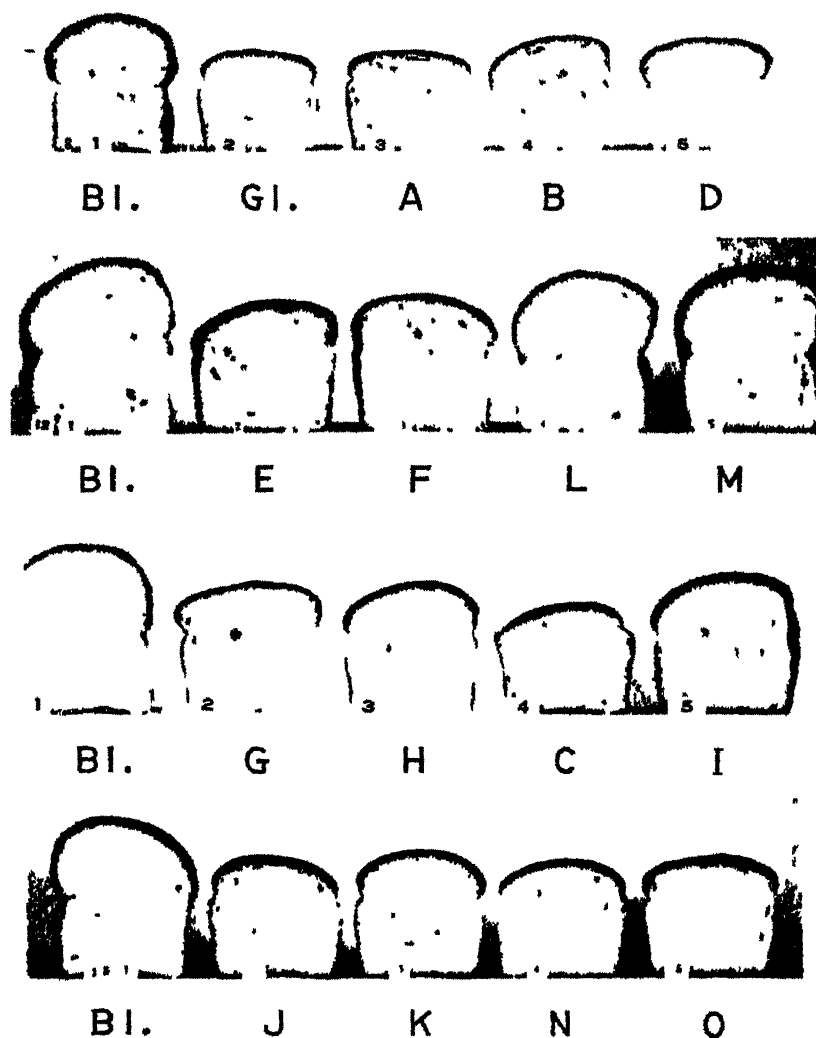


Fig 1. Effect of adding 3% (flour basis) of various dried nonviable yeasts, untreated, to the bread formula.

Bl = blank, no dry yeast added

Gl = glutathione added, 15 mg percent.

addition of 15 mg percent of glutathione (GSH). There is considerable variation in the effect of various dry yeasts on the bread. Only

yeasts *L* and *M* gave loaves classified as satisfactory. The next step was to investigate the possibilities of improving the breadmaking properties of the yeasts producing poor loaves.

Because the low-scoring loaves had the characteristics of bread requiring oxidizing agents, experiments were conducted along these lines. The effect of oxidizing agents in dough fermentation appears much later than the softening effect of dry yeast. For this reason, the use of oxidizing agents in the bread formula did not prevent the dough softening in the mixer; however, the softening action occurring in the mixer, in most instances, appeared to be reversible when the oxidizing agent had exerted its effect as fermentation progressed. A more direct method of eliminating the reducing substances in the dry yeast was to treat the yeast in a slurry (1 part yeast, 2 parts water) with the oxidizing agents before adding to the dough batch. Several common oxidizing agents, potassium bromate, potassium iodate, sodium chlorite, and hydrogen peroxide, were used in varying amounts with yeast *G*, which represented a yeast having an average dough-softening effect in the mixer. Hydrogen peroxide and sodium chlorite both gave rapid and effective oxidation. Conducting all treatments at room temperature (20°–25°C), the use of sodium chlorite to the extent of 1 mg per gram of dry yeast for 20 to 30 minutes before mixing gave optimum improvement for the average dry yeast. This rate of treatment is obviously not the optimum degree of oxidation for this entire lot of samples, which varied considerably in their dough-softening properties in the mixer, but this level proved to be a good working basis for improving all samples needing treatment.

All of the yeasts not giving a satisfactory score, when used at the 3% level, were treated with sodium chlorite in the manner already described. The baking results are shown in Figure 2 and Table I. This treatment gave a very definite loaf score improvement for all yeasts except *C*. Yeasts *A*, *B*, *E*, *F*, *J*, *O*, and *N* gave loaves that were acceptable, but could not be rated good. Varying the amounts of chlorite treatment did not give the desired improvement with these yeasts.

With yeast *C*, the loaf score decreased as a result of this treatment. When treated with chlorite it produced dough that became progressively softer during fermentation, similar to the effect of adding papain to a dough formula. This progressive softening was slightly more pronounced after the chlorite treatment than when used as received. The response of this sample likewise was not improved by varying the amount of the chlorite treatment. Because of the progressive softening of this dough during fermentation, and because the iodine titration



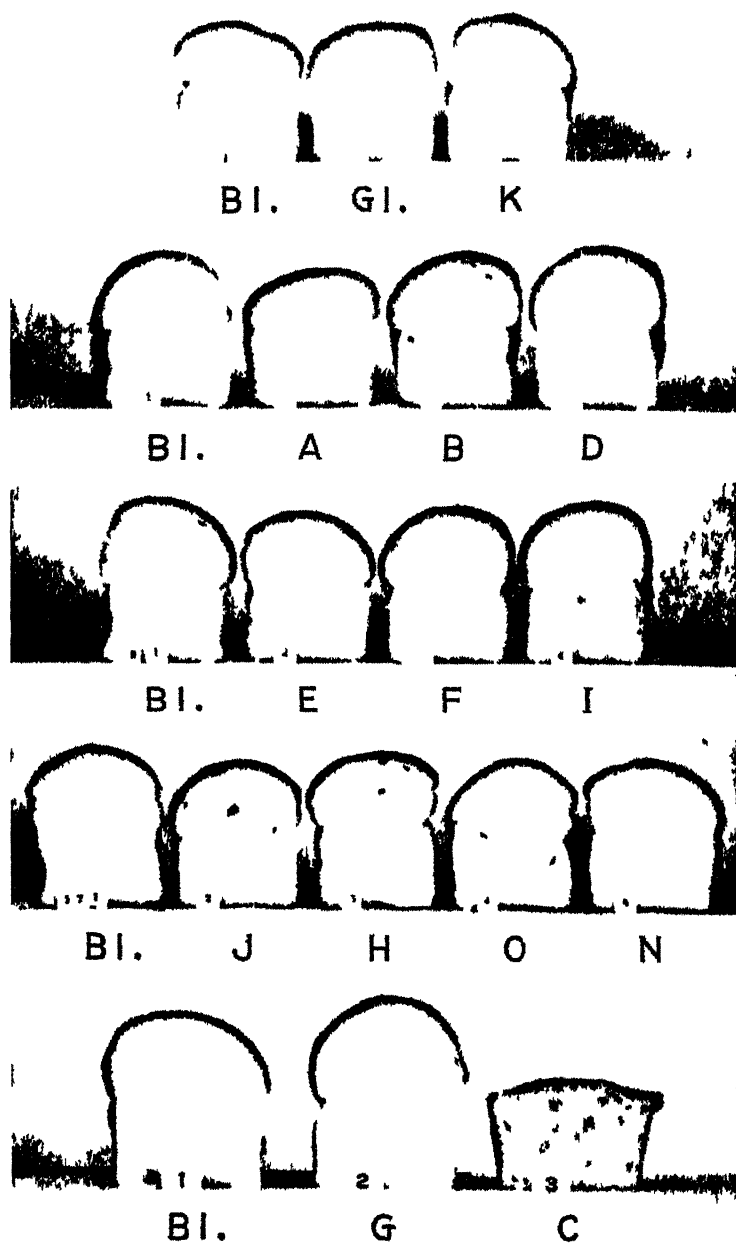


Fig 2 Effect of adding 3% (flour basis) of poor baking nonviable dried yeasts after the yeast had been treated with sodium chlorite (1 mg per gram)

BI = blank, no dry yeast added

GI = 15 mg percent oxidized with 4 mg sodium chlorite

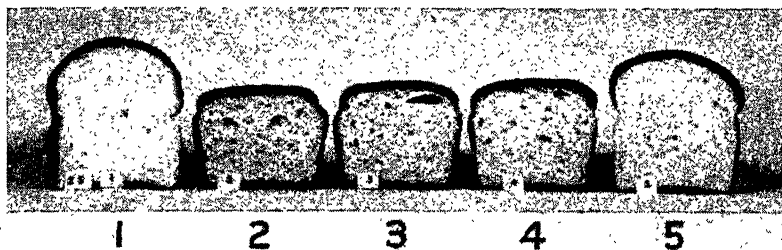


Fig. 3. Showing the development of baking improvement in a dry yeast originally giving very poor baking results.

1. Blank, no dry yeast in formula.
2. 3% dry yeast C added as received.
3. 3% dry yeast C added after treatment with sodium chlorite (1 mg per gram) 30 minutes before dough mixing.
4. 3% dry yeast C added after boiling 1 hour before adding to the dough mixer.
5. 3% dry yeast C added to the mixer after boiling 1 hour and subsequent treatment with sodium chlorite (1 mg per gram).

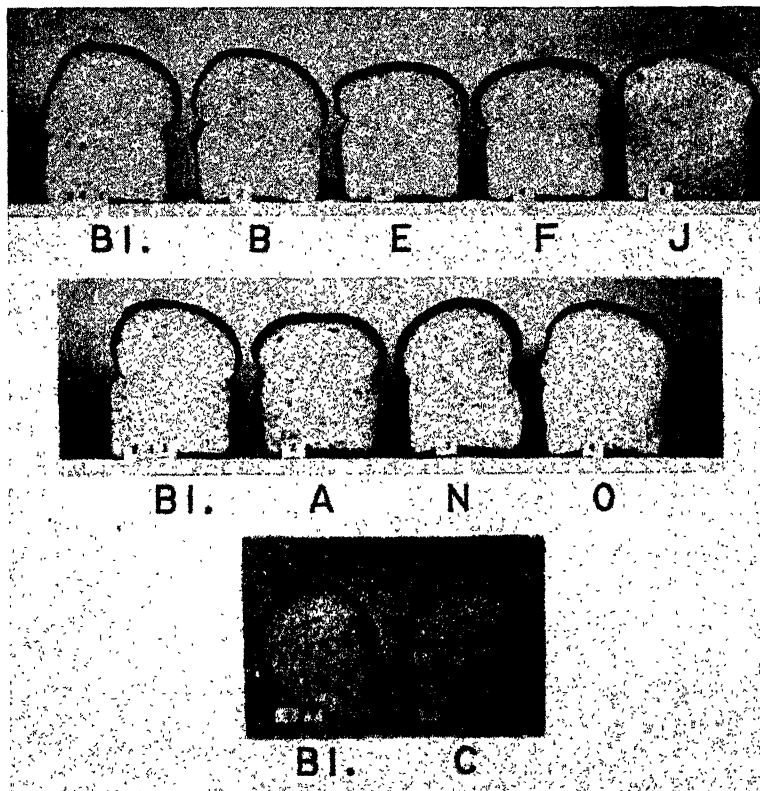


Fig. 4. Effect of treating poor baking nonviable dry yeasts with 1 hour boiling and subsequent sodium chlorite treatment (1 mg per gram) before inclusion in the bread formula. 3% dry yeast (flour basis) in formula.

Bl. = blank, no dry yeast in formula.

value<sup>3</sup> (as a measure of reducing substances) was not increased on heating this yeast, the effect was assumed to be enzymatic in character. Heat treatment was therefore employed to correct this condition. Figure 3 shows the result of both chlorite and heat treatment used separately and in combination on yeast *C*. The combined heat and chlorite treatment was necessary to produce acceptable bread with this yeast. This combined heating and chlorite treatment was next applied to all yeasts that did not give a loaf scoring "good" by the sodium chlorite treatment only. Figure 4 and Table I show that the combined treatment gave satisfactory bread with the remainder of the dry yeast samples.



Fig. 5. Results of using 5% properly treated dry yeasts in the bread formula. These yeasts differed greatly in their original functional behavior in the dough.

BI No dry yeast added.

L Yeast *L* with no treatment.

G Yeast *G* with sodium chlorite treatment only (1 mg per gram).

C Yeast *C* with boiling and sodium chlorite treatment.

Among the yeast samples used in these experiments three types of reactions in bread dough were encountered. Samples such as yeast *L* could be used at the 3% level as received without seriously affecting the loaf score. Others, such as yeast *G*, responded to the oxidizing (sodium chlorite) treatment to give improved baking results, while still other samples, such as yeast *C*, required heating and oxidizing treatment to produce the desired results. Yeasts representing each of these types were given their required treatment and used in bread to the extent of 5% (flour basis). The results indicated that, from the standpoint of dough-handling properties and factors related to gluten structure in the loaf, as evidenced by satisfactory loaf volume, grain, and texture (Figure 5) it is possible to use this amount of properly treated dry yeast in bread. The effects on the dough, however, were

<sup>3</sup> The iodine titration method used yeast equivalent to 1 g dry yeast to which was added 10 ml water (20°–25°C) in a 15 ml centrifuge tube. The suspension was agitated thoroughly for 1 minute and let stand for 10 minutes, then centrifuged. Five ml of the supernatant was diluted to about 50 ml with water, then 1 ml 1N H<sub>2</sub>SO<sub>4</sub> and 1 ml 2% starch solution were added. This mixture was titrated directly with .01 N iodine solution and the titration values calculated to milligrams of iodine per gram of dry yeast.

such as to indicate that this rate of use approaches the maximum even for a properly treated dry yeast.

In experiments where living yeast cells were made nonviable by heating, freezing, or mechanical treatment, there was a pronounced increase of reducing substances as measured by an iodine titration of an acidulated water extract of the yeast. The ordinary drying of yeast also causes a great increase in the iodine titration values of the water extract of the yeast. It appears that any means used to render yeast cells nonviable releases these reducing substances, which are similar to GSH groups in their effect on bread dough. Viable yeast can be made nonviable by heating to pasteurization temperatures, and with the reducing substances then released from the yeast cells they can be oxidized with some active oxidizing agent such as sodium chlorite or hydrogen peroxide before drying. When these substances have been properly released and oxidized the yeast can then be dried without further release of such materials.

The iodine titration values show a fairly good relation with the ultimate baking results of the untreated yeast (see Table I). In Figure 1, loaves *E*, *A*, *L*, and *M*, representing the poorest and best of the yeasts, correspond to iodine titration values of 5.9 mg, 5.6 mg, 1.1 mg, and 2.4 mg. In other series of yeast samples, a preliminary selection of the good and poor baking samples could be made by using this simple test. A test of this type with the added refinement of protein precipitation might be a simple, reliable control method for appraising the bread-baking value of nonviable dry yeast, particularly since the major dough impairing factors in dry yeast appear to be of the nature of reducing substances.

The behavior of these dry yeast samples, particularly sample *C*, which was dried in a low temperature process, suggests that in some processes proteolytic enzymes may survive the drying although the yeast loses its fermenting ability and viability. By elevating the heat treatment temperature in the yeast slurry to boiling, the proteolytic enzymes can be destroyed and the reducing substances which are released can then be oxidized before drying. This affords a practical procedure for eliminating both the reducing and enzymatic factors in producing nonviable dry yeast suitable for incorporating in bread dough.

This paper has been concerned only with the functional behavior of nonviable dry yeast as an ingredient in bread doughs. It is of interest to know how the recommended treatments for making yeast useful as a bread ingredient affect its nutritional values. Two yeast samples properly treated so that 5% (flour basis) could be added to

the bread gave the following average enrichment level with respect to some of the vitamin B-complex factors:

	Thiamine mg/lb	Riboflavin mg/lb	Niacin mg/lb
Control	0.25	0.27	3.6
Bread including 5% dry yeast	2.72	.78	8.8

Although these data are not extensive, it is evident that the treatment of the yeast does not destroy its value as a vitamin supplement for bread.

### Summary

Commercial nonviable dry yeasts vary considerably in the extent to which they affect bread dough when they are used in the bread formula.

Nonviable dry yeasts having a deleterious effect in bread dough can be improved by proper oxidizing treatment, or heating and oxidizing treatment, before being incorporated in the dough.

Two independent factors appear to cause nonviable dry yeast to soften bread doughs. The predominating factor behaved as though it consisted of reducing substances, and the second factor behaved as though it were enzymatic in character. In most of the dry yeasts the reducing substances were the predominant dough-softening factor, and an iodine titration of the water extract was found to be useful in predicting the baking value of the dry yeast.

These studies suggest that dry yeast of improved functional bread-baking qualities can be produced by heating the yeast to boiling temperatures before drying to release the reducing substances from the cell and to destroy proteolytic enzyme activity. The released reducing substances can then be oxidized before drying to produce a yeast of improved baking value.

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# INVESTIGATION OF THE PROXIMATE CHEMICAL COMPOSITION OF THE SEPARATE BRAN LAYERS OF WHEAT<sup>1</sup>

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Although the proximate composition of commercial bran is well known (Bailey, 1944), little information is available on the proximate chemical constitution of the individual layers of cells which compose bran. Goncalves (1944) gives data in which the bran was divided into (1) epidermis and cross layers, (2) testa and hyaline, and (3) aleurone. No method was given for the separations. Techniques by which individual bran layers may be separated from each other have hitherto been of a mechanical nature and too tedious and time-consuming to provide sufficient material for analysis.

Preliminary work in this laboratory led to a process for separating the bran layers from each other by a combination of physical and chemical methods. The process was applied to yield sufficient material for quantitative determinations of moisture, ash, protein, fat, crude fiber, cellulose, and pentosans. The results are presented in this paper.

## Materials and Methods

Four commercial blends of wheat were employed. Sample 1 was a hard red spring blend, used for the production of a family flour, while sample 2 was a hard red spring blend used in producing a bakers' flour. Sample 3 was a Michigan white wheat, while sample 4 was an Ohio soft red winter wheat.

The individual bran layers will be designated as shown in Figure 1. The epidermis was removed by suspending the wheat in water and stirring in a Waring Blendor equipped with rubber beaters. It was necessary to reduce the speed by means of a variable resistance. This treatment removed the epidermis completely except in the crease. The cross layer and testa were next removed as follows. The air-dried peeled wheat was soaked in a nearly saturated solution of sodium hydroxide in 95% ethyl alcohol overnight, the supernatant liquid poured off, and 95% ethyl alcohol added. The testa layer was

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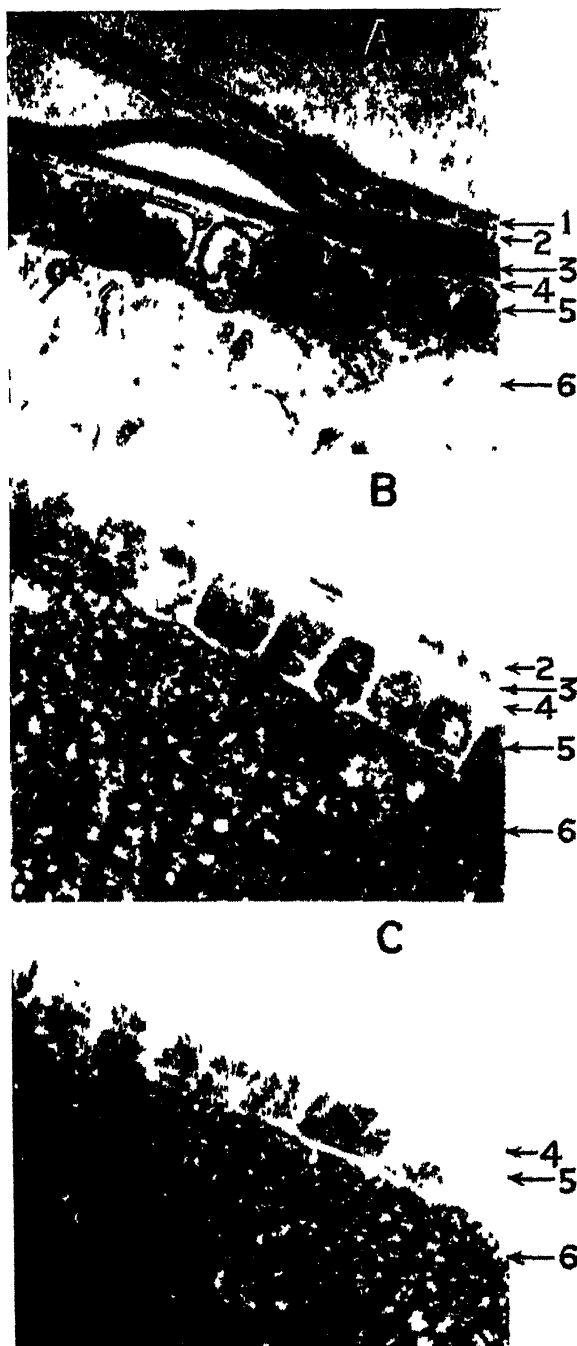


Fig 1. Photomicrographs of wheat cross sections after successive removal of bran layers.  
Magnified about 215 times.  
A. Original wheat. B. After removal of epidermis. C. After removal of cross layers and testa.  
1. Epidermis. 2. Cross layer cells. 3. Testa layers. 4. Hyaline.  
5. Aleurone. 6. Starchy endosperm.

then separated by stirring the grain with more 95% ethyl alcohol in the Waring Blendor at reduced speed. Each preparation was removed by filtration and air dried. Microscopic examination of the grain showed that the bran layers were removed to the hyaline layer (Figure 1).

The de-branned wheat was placed in a large Buechner funnel and washed once with 95% alcohol, then once with water, followed by a wash with dilute hydrochloric acid, and finally by another water wash before being air dried.

After air drying, the de-branned wheat was tempered to a moisture content of 16% and milled on an experimental mill to obtain samples of endosperm and of aleurone with the adhering hyaline. The latter preparation was contaminated with considerable endosperm, and, consequently, corrections, based upon its starch content, were applied to the determinations.



Fig. 2. Photomicrograph of epidermis preparation. Only epidermis cells appear to be present. Magnified about 50 diameters.

Microscopic examination of the various preparations (Figures 2, 3, and 4) indicated that the testa was contaminated with cross layer and epidermis cells. Consequently, a correction was made as follows. A small amount of the dry preparation was placed on a blood-counting chamber, and evenly distributed with a spatula. Then the length and breadth of every particle occurring within the marked area were measured with a microscope containing a scale in the eyepiece. A thickness factor for each layer was determined by measuring the relative thickness of each layer in cross section of wheat grains with the same scaled eyepiece. These three factors were then multiplied together to obtain the relative volume of each particle. The sum of the volumes for each of the three fractions divided by the total volume



was assumed to represent the percentage of the fraction present. The results of the testa fraction were then corrected for the percentage of each contaminating layer found by this procedure.



Fig. 3 Photomicrograph of cross layer preparation. Only cross layer cells are present. Magnified about 50 diameters.



Fig. 4 Photomicrograph of testa preparation. Epidermis cells (a) cross layer cells (b) and testa cells (c) are present in the preparation. Magnified about 50 diameters.

To check the operations during the removal of various layers, proximate analyses were made on the whole wheat and also upon the portion left after removal of each layer. The latter figures were compared with a calculated value which the preparation should have on the basis of the proximate analysis of the removed layer.

Moisture, protein, total ash, crude fat (ether extract), crude fiber, pentosans, and starch (by Rask's procedure) were determined as described in *Cereal Laboratory Methods* (4th ed., 1941). Cellulose was determined by the method of Crampton and Maynard (1938).

## Results

The percentages of each layer found in whole wheat and bran after making the corrections described are shown in Table I.

TABLE I  
PERCENTAGE DISTRIBUTION OF TOTAL DRY MATTER OF BRAN  
AMONG THE INDIVIDUAL LAYERS

	Sample 1		Sample 2	
	Per cent of whole wheat	Per cent of bran	Per cent of whole wheat	Per cent of bran
Epidermis	3.9	26.7	3.9	27.1
Cross layer	0.9	6.2	0.9	6.2
Testa	0.5	3.4	0.7	4.9
Aleurone and hyaline	9.3	63.7	8.9	61.8
Total	14.6	100.0	14.4	100.0

The epidermis and the hyaline-aleurone preparation constitute the majority of the dry matter of the bran (about 27% and 62% respectively). The amounts of testa and cross layers are comparatively small. The sum of the percentages of the individual bran layers indicates the percentage of bran in wheat. The brans of these wheats amounted to about 14.5% of the kernel. This figure corresponds with that given by the Wheat Flour Institute (1940), but is considerably higher than the value of 8% given by Briggs (1935). The experimental values are also somewhat higher than those suggested by Miller (1941) who estimates the content to be somewhat over 12%. Jago and Jago (1921) give the following figures from Mege Mouris:

<i>Percentage of entire kernel</i>	<i>%</i>
Epicarp	0.5
Mesocarp	1.0
Endocarp	1.5
Testa and hyaline	2.0

The epicarp and mesocarp taken together correspond to the portion referred to in this paper as epidermis, and the endocarp corresponds to the cross layer fraction. While the amount of the epidermis found in this study was greater than that reported by Mege Mouris, his figure for the cross layer fraction is higher. The hyaline layer was not separated from the aleurone layer in the present work and therefore it is not possible to compare directly his estimation for the constructed percentage of testa and hyaline.

If the figure of 2% for testa and hyaline given by Mege Mouris is assumed to be correct, then by subtracting the average value found for testa in the present work, the figure of 1.4% is obtained for the

TABLE II  
PROXIMATE CHEMICAL COMPOSITION OF THE DIFFERENT PARTS OF THE WHEAT KERNEL<sup>1</sup>

Constituents	Whole wheat				Bran layers					Hand-picked hyaline	Starchy endosperm		Hand-picked starchy endosperm
	Original	Minus epidermis		Minus cross layers, testa	Epl-dermis	Cross layers	Testa	Hyaline-aleurone	Hand-picked hyaline-aleurone		Endosperm		
		Anal.	Calc.							Anal.	Calc.		
												%	%
	%	%	%	%	%	%	%	%	%	%	%		
Total ash	1.87	1.88	2.19	1.62	1.41	13.8	19.3	4.9	—	0.70	0.92	—	
Sample 1	1.88	1.95	1.90	1.69	1.35	15.7	23.5	5.7	1.98	0.74	0.90	—	
Sample 2	2.03	—	—	—	1.38	13.1	14.6	7.5	—	0.66	—	—	
Sample 3	2.24	—	—	—	1.53	12.6	14.6	12.6	—	0.70	—	0.60	
Sample 4													
Crude protein													
Sample 1	15.0	15.7	15.9	15.8	5.2	10.9	13.9	33.4	—	13.9	13.6	—	
Sample 2	16.1	16.4	16.5	16.4	3.6	11.2	22.7	37.6	19.5	14.2	13.8	—	
Sample 3	11.4	—	—	—	—	—	—	30.6	—	8.5	—	—	
Sample 4	14.1	—	—	—	—	—	—	29.3	29.5	8.7	—	10.3	
Crude fat													
Sample 1 <sup>2</sup>	2.2	2.3	2.2	2.3	1.2	0.6	0.0	8.8	—	1.0	1.3	—	
Sample 2 <sup>2</sup>	2.3	2.3	—	2.4	0.8	0.4	0.2	7.0	—	1.0	0.9	—	
Sample 2 <sup>3</sup>	3.0	—	2.4	—	—	—	—	10.6	—	—	—	—	
Sample 3 <sup>3</sup>	3.1	—	—	—	—	—	—	11.5	—	1.9	—	—	
Sample 4 <sup>3</sup>	3.1	—	—	—	—	—	—	8.8	—	2.2	—	2.8	

<sup>1</sup> Moisture-free basis.<sup>2</sup> Ether extract method.<sup>3</sup> Acid hydrolysis method.

TABLE II—Continued

Constituents	Whole wheat						Bran layers					Hand-picked hyaline	Starchy endosperm		Hand- picked starchy endosperm
	Original	Minus epidermis		Minus epidermis, cross layers, testa		Epi- dermis	Cross layers	Testa	Hyaline- aleurone	Hand- picked hyaline- aleurone	Endosperm				
		Anal.	Calc.	Anal.	Calc.						Anal.	Calc.			
													%	%	%
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
Crude fiber	Sample 1	3.1	3.0	2.3	1.0	1.5	28.2	21.4	1.2	6.1	—	—	0.3	0.4	—
	Sample 2	2.6	1.6	1.9	1.0	1.4	27.1	19.9	1.4	7.0	—	—	0.2	0.4	—
	Cellulose														
Cellulose	Sample 1	2.5	1.4	1.5	1.0	1.3	32.4	22.8	0.0	6.5	—	—	0.3	0.4	—
	Sample 2	2.1	1.7	1.4	1.0	1.1	31.8	22.9	0.0	6.5	—	7.0	0.3	0.4	—
	Sample 3	2.2	—	—	—	—	—	—	—	5.1	—	—	0.2	—	—
	Sample 4	2.6	—	—	—	—	—	—	—	4.2	3.3	—	0.2	—	0.2
Pentosans	Sample 1	6.5	5.6	5.7	5.4	5.2	34.0	30.3	18.2	33.7	—	—	3.5	4.4	—
	Sample 2	6.9	5.8	6.0	5.3	5.6	37.9	29.2	16.0	25.9	—	46.1	3.5	3.1	—
	Sample 3	7.2	—	—	—	—	—	—	—	30.9	—	—	3.0	—	—
	Sample 4	7.8	—	—	—	—	—	—	—	25.8	27.5	—	3.2	—	3.3
Starch	Sample 1	63.1	—	—	—	—	—	—	—	—	—	—	78.9	—	—
	Sample 2	61.3	—	—	—	—	—	—	—	—	—	—	78.1	—	—
	Sample 3	68.4	—	—	—	—	—	—	—	—	—	—	82.7	—	—
	Sample 4	64.1	—	—	—	—	—	—	—	—	—	—	83.8	—	80.2

hyaline layer. By subtracting this value from the average value obtained for the hyaline-aleurone preparation, the figure of 7.7% is obtained for the aleurone layer. The total of the layers reported upon by Mege Mouris amounts to 5%. From the figure given by Briggs for bran, this would only allow the aleurone to be about 3% of the whole wheat, a much lower value than that found in this study.

Results of the proximate chemical analyses are shown in Table II.

The distribution of the different constituents in the individual bran layers and in the starchy endosperm were calculated from the data recorded in Tables I and II and are summarized in Tables III and IV.

TABLE III

AVERAGE PERCENTAGE DISTRIBUTION OF ASH, PROTEIN, FAT, CRUDE FIBER, CELLULOSE, AND PENTOSANS OF THE TOTAL BRAN FOUND IN THE INDIVIDUAL BRAN LAYERS<sup>1</sup>

Layer	Ash	Protein	Crude fat	Crude fiber	Cellulose	Pentosans
	%	%	%	%	%	%
Epidermis	6.8	4.8	4.1	58.1	61.2	34.8
Cross layers	16.5	2.6	0.6	9.6	9.7	6.4
Testa	16.2	2.9	0.1	0.0	0.0	2.4
Hyaline and aleurone	60.5	89.7	94.2	32.3	29.1	56.4

<sup>1</sup> Data for samples 1 and 2 averaged together. Obtained by calculation from Tables I and II.

TABLE IV

AVERAGE PERCENTAGE DISTRIBUTION OF ASH, PROTEIN, FAT, CRUDE FIBER, CELLULOSE, AND PENTOSANS OF THE TOTAL GRAIN FOUND IN DIFFERENT PARTS OF THE WHEAT KERNEL<sup>1</sup>

Layer	Ash	Protein	Crude fat	Crude fiber	Cellulose	Pentosans
	%	%	%	%	%	%
Epidermis	2.8	1.2	1.8	37.8	55.2	20.8
Cross layers	7.0	0.6	0.2	6.2	8.7	3.8
Testa	7.0	0.7	0.0	0.0	0.0	1.4
Hyaline-aleurone	25.7	20.8	32.0	21.0	26.2	33.8
Starchy endosperm	32.8	77.3	38.6	7.7	12.1	44.8
TOTAL	75.3	100.6	72.8	76.7	102.2	104.6

<sup>1</sup> Data for samples 1 and 2 averaged together.

*Ash.* The results in Table II present a fairly clear picture of the ash content of the bran layers. Since both the cross layer and testa fractions had absorbed sodium hydroxide in the removal process, the ash contents of these fractions were corrected by making sodium determinations using the uranyl acetate precipitation method (A. O. A. C. official method, 1940). On the basis of preliminary work the preparations were assumed naturally to contain 1,000 p.p.m. sodium and the excess sodium contents, calculated as sodium oxide, were deducted from the original ash to give the figures recorded in Table II.

The average ash content of the various fractions is as follows:

	%
Epidermis	1.4
Cross layer	13.0
Testa	18.0
Aleurone-hyaline preparation	6.0
Endosperm (starchy)	0.7

The ash content of the hyaline-aleurone preparation is of the same order as that of whole bran, while the ash content of the endosperm corresponds to that of clear flour. The ash contents of the cross layer and testa fractions are very high.

The variation in the ash contents of the testa and hyaline-aleurone fractions of the different wheat samples was quite large. The ash content of the hyaline-aleurone of sample 4 was especially high, but this figure was verified in the corresponding hand-picked sample. However, because of the small number of samples, it is not possible to state any definite trend in wheat types. The ash content of one sample (No. 4) of hand-picked hyaline layer was much lower than that of the corresponding hyaline-aleurone sample, indicating that the ash of the latter is concentrated largely in the aleurone layer. The ash contents of the aleurone layers should then be higher than those recorded for the hyaline-aleurone combinations.

As shown in Tables III and IV, the hyaline-aleurone preparation contains almost 60% of the total ash of the bran, and about 25% of the ash of the whole wheat.

*Crude Protein.* The average protein contents of the different bran layers expressed on the dry basis are approximately as follows:

	%
Epidermis	4
Cross layer	11
Testa	18
Hyaline-aleurone preparation	33
Starchy endosperm	11

These data were verified by a qualitative microchemical study *in situ* in which the testa layer and the aleurone cell contents were shown to contain considerable protein. One of the most striking facts is the high protein content of the hyaline-aleurone preparation. Since the protein content of the sample of hyaline (which was shown microscopically still to contain some aleurone cells) is lower than that of the corresponding hyaline-aleurone combination, the protein content of the aleurone layer must be higher than those recorded for the hyaline-aleurone preparations.

The hyaline-aleurone preparation constitutes about 90% of the protein of the whole bran, and slightly over 20% of the protein of the whole wheat.

*Crude Fat.* Of the bran layers, only the hyaline-aleurone preparation contains more than a negligible amount of crude fat. Of the two layers, qualitative microchemical studies indicated that the aleurone probably contains the bulk of this constituent. The acid hydrolysis method for fat yields considerably more fat from the hyaline-aleurone preparation than does the ether extraction method. This is perhaps due to the heavy cell walls of the aleurone which are hydrolyzed by the former method. The hyaline-aleurone preparation contains about 94% of the ether extract of the bran, and about 32% of the ether extract of the whole grain, while the starchy endosperm contains about 38% of the ether extract of the whole grain.

*Crude Fiber and Cellulose.* As one would suspect, the pictures for crude fiber and cellulose are roughly parallel to each other. The average percentages of crude fiber and cellulose are as follows in round numbers:

	Crude fiber %	Cellulose %
Epidermis	28	32
Cross layer	21	23
Testa	1.3	0.0
Hyaline-aleurone preparation	6.5	6.5
Endosperm (starchy)	0.3	0.3

These data were again verified by qualitative microchemical data which indicated that cellulose is high in the epidermis, cross layers, and hyaline cell walls, present in the aleurone cell walls, but almost non-existent in the testa layer. In the case of white wheat, however, microchemical studies indicated that a small amount of cellulose does occur in the cell walls of the testa. The apparent absence of this constituent in the testa may be due to the inaccuracy of the correction procedure as previously described.

Table III shows that the epidermis contains 61% and the hyaline-aleurone preparation 29% of the cellulose in bran. When referred to the whole grain, the epidermis contains 55%, the hyaline-aleurone preparation 25%, and the endosperm 12% of the cellulose.

*Pentosans.* The pentosans seem to be uniformly distributed throughout the bran, as can be seen from the following average results:

	%
Epidermis	35
Cross layer	30
Testa	17
Hyaline-aleurone preparation	30
Starchy endosperm	3.5

It is noteworthy that the sample of hand-picked hyaline was very high in pentosan content.

The epidermis contains 35% and the hyaline-aleurone preparation 56% of the pentosan content of the bran (Table III), whereas the epidermis contains 21%, the hyaline-aleurone preparation 34%, and the endosperm about 44% of the pentosans of the whole wheat (Table IV).

### Discussion

The results indicate that the nutritive value of bran resides largely in the hyaline-aleurone preparation, as this fraction contains the bulk of the ash, protein, and fat of the bran, and a large portion of the same constituents in whole wheat. The other layers contain little nutritive value, but the epidermis and cross layers would serve as a good source of furfural because of their high pentosan content.

Over 50% of the mineral content of whole wheat is contained in bran. Comparison of the proximate chemical analysis of the residual wheat after removal of the epidermis, cross layer, and testa, and the starchy endosperm recorded in Table II, with the calculated values indicates that some ash was lost in the process of removing the cross layer and testa cells. On the other hand, the experimental values for protein agree with the calculated ones within experimental error. For many of the other constituents, this is also true.

The sum of the ash, protein, fat, cellulose, and pentosan percentages for each individual layer is less than 100. Cutin, suberin, pectin compounds, phytin, and lignin were not determined, but were present in some layers as indicated by microchemical tests.

### Summary

The various layers of wheat bran were separated by a physical and chemical process, and subjected to proximate chemical analysis. The total bran was found to be about 14.5% of the whole wheat, of which about 3.9% was epidermis, 0.9% cross layers, 0.6% testa, and 9.0% hyaline and aleurone. Wheat epidermis contained about 1.4% ash, 4% protein, 1% fat, 32% cellulose, and 35% pentosans. The cross layers contained about 13% ash, 11% protein, 0.5% fat, 23% cellulose, and 30% pentosans. The testa layers contained about 18% ash, 15% protein, no cellulose, no fat, and 17% pentosans. A hyaline-aleurone preparation contained about 5% ash, 35% protein, 7% fat, 6% cellulose, and 30% pentosans.

The starchy endosperm contained about 0.7% ash, 14% protein, 1% fat, 0.3% cellulose, and 3.5% pentosans.

The hyaline-aleurone preparation contained about 60% of the ash, 90% of the protein, 94% of the fat, 56% of the pentosans, and 29% of the cellulose in whole bran. All figures are expressed on the dry basis.



Of the botanical layers which occur in commercial bran, the aleurone layer appears to be of paramount importance from a nutritive viewpoint.

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### MULTIPLE BLEACHING OF FLOUR. IV. BLEACHING EFFICIENCY IN SYSTEMS INVOLVING CHLORINE DIOXIDE AND ANOTHER BLEACHING GAS<sup>1</sup>

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The purpose of this paper is to report experimental results which show the best way to utilize chlorine dioxide in combination with another bleaching gas in the multiple bleaching of flour.

In recent years much interest has been evidenced in the evaluation of chlorine dioxide as a flour bleaching reagent. This reagent was shown by Ferrari, Hutchinson, Croze, and Mecham (1941) to be quite similar in its flour bleaching action to nitrogen trichloride. To bring about a given amount of color removal or to mature a flour from the

<sup>1</sup> Paper No. 70, Journal Series, General Mills, Inc., Research Department.

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baking standpoint, only from one-half to two-thirds as much chlorine dioxide is needed as in the case of nitrogen trichloride. Until recently the methods for making chlorine dioxide were inadequate for providing the fine control needed in applying this gas for flour bleaching purposes. However, commercial equipment has now been manufactured by the Mathieson Alkali Works, Inc., based on the process developed by Hutchinson and Mecham (1943). Adequate control of chlorine dioxide for flour bleaching purposes can be obtained by this method.

The first three papers of this series by the present authors and Mecham (1945) dealt with repeated bleaching treatments of flour with a single gaseous reagent and the effects of agitation on bleaching efficiency, multiple bleaching with nitrogen peroxide and another gas, and multiple bleaching with nitrogen trichloride and another gas, respectively. Multiple bleaching has been defined as the treatment of flour by repeated applications of a single gaseous reagent or by the simultaneous or sequential treatment of flour with two or more gaseous bleaching reagents.

Some multiple bleaching treatments involving the use of chlorine dioxide with such other bleaching gases as chlorine, hypochlorous acid, nitrogen trichloride, and nitrogen peroxide have been covered by a patent to Ferrari and Hutchinson (1943).

### Apparatus and Procedure

The agitating equipment and methods for the generation and metering of the bleaching gases have been briefly described by Ferrari, Hutchinson, and Mecham (1945), in the first paper of this series and are dealt with in more detail by Hutchinson, Derby, and Ferrari (1947).

### Experimental

*Multiple Bleaching with Chlorine Dioxide and Chlorine.* Experiments were conducted in which chlorine dioxide and chlorine were used in combination in the batch scale bleaching of patent flour. In order to ascertain the best way in which to utilize these reagents in multiple bleaching treatments, they were applied to the flour both simultaneously and sequentially. Typical examples of such treatments are shown in Table I. It is seen that a substantial increase in color removal is gained by applying the chlorine to the flour first, followed by the chlorine dioxide. The application of the two reagents in the reverse order or simultaneously resulted in much less color removal. Judging from an extensive experience with many flours, it is probable that it would take between 0.016 and 0.032 ounces of benzoyl peroxide per barrel to reduce the carotene content of this flour from 0.98 to 0.80 p.p.m. From the economic standpoint such a saving in benzoyl

peroxide due to the application of chlorine and chlorine dioxide in the preferred order is quite significant.

Although some difference in effect on pH may be noted in Table I due to the method of application of the two gases, it is not regarded as significant.

TABLE I  
CHLORINE DIOXIDE AND CHLORINE APPLIED TO A PATENT FLOUR  
SIMULTANEOUSLY OR SEQUENTIALLY

Bleaching treatment per bbl flour	pH	Carotene <sup>1</sup> p.p.m.
Unbleached patent flour	6.01	2.20
2 g NCl <sub>3</sub> plus 0.048 oz benzoyl peroxide	6.03	0.66
0.8 g ClO <sub>2</sub>	6.03	1.27
21 g Cl <sub>2</sub>	5.91	1.32
Simultaneous: 0.8 g ClO <sub>2</sub> and 21 g Cl <sub>2</sub>	5.84	0.98
Sequential: 0.8 g ClO <sub>2</sub> followed by 21 g Cl <sub>2</sub>	5.81	0.99
21 g Cl <sub>2</sub> followed by 0.8 g ClO <sub>2</sub>	5.74	0.80

<sup>1</sup> Flour pigment content expressed as carotene.

From the baking standpoint this bleaching combination offers some interesting possibilities due to the lowering of pH by the chlorine and the maturing action of the chlorine dioxide. Practically all of the flour samples which have been subjected to multiple bleaching treatments have been baked in one or more experimental or commercial baking procedures. Generally speaking, it has been found that the flour bleached with the preferred order of application of the reagents from the color removal standpoint has possessed at least as good baking characteristics as flours bleached with the same quantities of the same reagents simultaneously or in the reverse order of application.

*Multiple Bleaching with Chlorine Dioxide and Hypochlorous Acid.* In the bleaching of flour with chlorine dioxide and hypochlorous acid, the greatest color removal is realized when the hypochlorous acid is applied first, followed by the chlorine dioxide. The reverse order of application of these reagents brings about less color removal. Typical examples illustrating these facts are shown in Table II. It can be seen that the advantage due to the preferred order of application of the reagents is rather slight in the case of the patent flour which was bleached by the two gases to the level of about 0.75 p.p.m. of residual carotene. In contrast to this result is the rather large difference in color removal with the two orders of application of chlorine dioxide and hypochlorous acid on the first clear flour shown in Table II. With some reagent combinations the advantages of the preferred order of application become quite slight at low residual carotene values in the bleached flour, although they are pronounced at high residual carotene values. With other reagent combinations the effects are pronounced at both high and low residual carotene values in the bleached flour.

TABLE II  
FLOUR BLEACHING WITH CHLORINE DIOXIDE AND HYPOCHLOROUS ACID  
IN BATCH SCALE EQUIPMENT—EFFECT OF ORDER OF APPLICATION  
ON COLOR REMOVAL

Bleaching treatment per bbl flour	Carotene p p m
Unbleached patent flour	2.41
2 g $\text{NCl}_3$ plus 0.048 oz benzoyl peroxide	0.75
1.1 g $\text{ClO}_2$	1.21
20 g $\text{HOCl}$	1.27
1.1 g $\text{ClO}_2$ followed by 20 g $\text{HOCl}$	0.77
20 g $\text{HOCl}$ followed by 1.1 g $\text{ClO}_2$	0.75
Unbleached first clear flour	2.94
5 g $\text{NCl}_3$	1.20
1.1 g $\text{ClO}_2$	2.32
1.4 g $\text{ClO}_2$	2.08
1.1 g $\text{ClO}_2$ followed by 15 g $\text{HOCl}$	2.27
15 g $\text{HOCl}$ followed by 1.1 g $\text{ClO}_2$	1.97
1.4 g $\text{ClO}_2$ followed by 25 g $\text{HOCl}$	1.65
25 g $\text{HOCl}$ followed by 1.4 g $\text{ClO}_2$	1.37

In papers I and II of this series, it has been shown that chlorine dioxide is preferably applied to the flour first, followed by nitrogen trichloride or nitrogen peroxide, to achieve the greatest color removal effect.

### Summary

The preferred orders of application of chlorine dioxide and other gaseous reagents in the multiple bleaching of flour are as follows:

Chlorine dioxide followed by nitrogen trichloride  
Chlorine dioxide followed by nitrogen peroxide  
Hypochlorous acid followed by chlorine dioxide  
Chlorine followed by chlorine dioxide

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# MULTIPLE BLEACHING OF FLOUR. V. BLEACHING EFFICIENCY IN SYSTEMS INVOLVING CHLORINE AND HYPOCHLOROUS ACID OR THREE GASEOUS REAGENTS<sup>1</sup>

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The purposes of this paper are to submit data on the bleaching of flour with various combinations of three gaseous reagents selected from the group consisting of chlorine, chlorine dioxide, nitrogen trichloride, and nitrogen peroxide; to show the effects of blending two flours subjected to multiple bleaching treatments with different combinations of gaseous reagents; and to summarize and integrate the data submitted in this series of papers on the multiple bleaching of flour with gaseous reagents.

Multiple flour bleaching treatments involving chlorine and nitrogen peroxide, nitrogen trichloride, and chlorine dioxide have been described in papers II, III, and IV, respectively, of this series by the present authors (1945, 1945, and 1947). In this report results with the combination of chlorine and hypochlorous acid will be considered.

Since such remarkable and unexpected results were obtained by multiple flour bleaching treatments involving two gaseous reagents, it was decided to ascertain the feasibility of using multiple bleaching treatments involving three gaseous reagents applied to the same flour. The blending of samples of the same flour bleached with multiple bleaching treatments involving different combinations of reagents appeared to offer more bleaching flexibility, particularly from the baking standpoint, and was accordingly investigated. Such blended flours may involve three or four gaseous reagents.

Some of the multiple bleaching treatments described in this paper are covered by patents issued to Ferrari and Hutchinson (1943 and 1944).

## Apparatus and Procedures

Methods for the generation and metering of the bleaching gases and the agitating equipment have been briefly described in the first paper of this series (Ferrari, Hutchinson, and Mecham, 1945). They are described in greater detail by Hutchinson, Derby, and Ferrari (1947).

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*Multiple Bleaching with Chlorine and Hypochlorous Acid.* Flour bleaching with combinations of chlorine and hypochlorous acid is best carried out by applying the two reagents in sequence rather than simultaneously. Examples of such treatments are shown in Table I.

TABLE I  
CHLORINE AND HYPOCHLOROUS ACID APPLIED TO A PATENT FLOUR  
SIMULTANEOUSLY OR SEQUENTIALLY IN BATCH SCALE APPARATUS

Description of bleaching treatment per bbl flour	Pekar or slick score	Carotene <sup>1</sup> p p m
Unbleached	Creamy yellow	2.84
2½ g NCl <sub>3</sub> plus 0.32 oz benzoyl peroxide	10	0.85
25 g HOCl	8 Creamy	1.48
0.75 oz Cl <sub>2</sub>	—	1.63
Simultaneously: 0.75 oz Cl <sub>2</sub> plus 25 g HOCl	8 Creamy	1.32
Sequentially: 25 g HOCl followed by 0.75 oz Cl <sub>2</sub>	9 Slightly creamv	1.10
Sequentially: 0.75 oz Cl <sub>2</sub> followed by 25 g HOCl	9 Slightly creamv	1.08

<sup>1</sup> Flour pigment content expressed as carotene

The order of application of the two reagents is immaterial, but either sequence is highly preferable to the simultaneous application of the two reagents. A substantial quantity of benzoyl peroxide, probably between 0.016 and 0.032 ounces per barrel of flour, would be needed to lower the carotene content from 1.32 to 1.10 p.p.m.; so the practical importance of applying chlorine and hypochlorous acid in sequence is quite apparent.

*Multiple Bleaching with Three Gaseous Reagents.* In the preceding papers of this series on the multiple bleaching of flour with gaseous reagents many different combinations of two reagents have been pointed out which produce excellent bleaching results from the color removal standpoint. The great flexibility in the bleaching process resulting from these many reagent combinations has greatly increased the possibilities of achieving optimum baking properties through the proper choice of reagents for each different flour.

As chlorine, nitrogen trichloride, chlorine dioxide, and nitrogen peroxide all have somewhat different effects on the baking properties of flour, it was thought that flexibility in bleaching treatments might be increased still more by the application of three gaseous reagents to the same flour.

Four possible ways of applying any three gaseous reagents to flour are as follows:

1. Simultaneous application of the three gases.
2. Sequential treatment with the three gases (six possible combinations).

3. Application of one gas followed by a simultaneous treatment with the other two gases (three possible combinations).
4. Simultaneous application of two gases followed by a treatment with the third gas (three possible combinations).

Thirteen different treatments are, therefore, possible for the application of any three gaseous reagents to flour.

Since the results with the simultaneous treatment of flour with two gases were found so uniformly poor compared to a preferred order of application of combinations of two gaseous bleaching reagents, the simultaneous treatment of flour with three gaseous reagents was not tried. However, methods 2, 3, and 4 above were all made the subject of extended experimentation.

TABLE II  
BLEACHING OF PATENT FLOUR WITH CHLORINE DIOXIDE, NITROGEN  
TRICHLORIDE, AND NITROGEN PEROXIDE APPLIED SEQUENTIALLY  
IN BATCH SCALE EQUIPMENT

Bleaching treatment per bbl flour	Carotene p.p.m.
Unbleached flour	2.20
0.8 g ClO <sub>2</sub> followed by 2 g NCl <sub>3</sub> followed by 1.5 g NO <sub>2</sub>	0.63
Sample after 0.8 g ClO <sub>2</sub> alone	1.42
Sample after 0.8 g ClO <sub>2</sub> followed by 2 g NCl <sub>3</sub>	0.69
0.8 g ClO <sub>2</sub> followed by 1.5 g NO <sub>2</sub> followed by 2 g NCl <sub>3</sub>	0.68
Sample after 0.8 g ClO <sub>2</sub> alone	1.38
Sample after 0.8 g ClO <sub>2</sub> followed by 1.5 g NO <sub>2</sub>	0.86
0.8 g ClO <sub>2</sub> followed by 1.5 g NO <sub>2</sub> followed by 0.75 oz Cl <sub>2</sub>	0.82
Sample after 0.8 g ClO <sub>2</sub> alone	1.36
Sample after 0.8 g ClO <sub>2</sub> followed by 1.5 g NO <sub>2</sub>	0.86
0.8 g ClO <sub>2</sub> followed by 0.75 oz Cl <sub>2</sub> followed by 1.5 g NO <sub>2</sub>	0.78
Sample after 0.8 g ClO <sub>2</sub> alone	1.40
Sample after 0.8 g ClO <sub>2</sub> followed by 0.75 oz Cl <sub>2</sub>	1.01

Typical examples of method 2 are shown in Tables II and III. In Table II several things may be noted. First it can be seen that chlorine dioxide was applied first in each of the bleaching treatments shown. The accuracy of the application of the chlorine dioxide treatments is shown in the residual carotene contents of 1.42, 1.38, 1.36, and 1.40 p.p.m. for the four different applications. The two cases in which 1.5 g nitrogen peroxide was applied following the chlorine dioxide both resulted in residual carotene values of 0.86 p.p.m., again showing the fine control that was achieved in the experimental bleaching techniques used.

In Table II it is seen that chlorine dioxide followed by nitrogen trichloride followed by nitrogen peroxide brought about more color

removal than chlorine dioxide followed by nitrogen peroxide followed by nitrogen trichloride. Also the application of chlorine dioxide followed by chlorine followed by nitrogen peroxide was more effective from the color removal standpoint than chlorine dioxide followed by nitrogen peroxide followed by chlorine. Table III shows that treatment of flour with nitrogen trichloride followed by chlorine followed by nitrogen peroxide was more effective than the application of nitrogen trichloride followed by nitrogen peroxide followed by chlorine. From these examples the generalization may apparently be made that

TABLE III  
BLEACHING OF PATENT FLOUR WITH NITROGEN TRICHLORIDE, NITROGEN  
PEROXIDE, AND CHLORINE APPLIED SEQUENTIALLY  
IN BATCH SCALE APPARATUS

Bleaching treatment per bbl flour	Carotene p.p.m.
2 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$ followed by 0.75 oz $\text{Cl}_2$	0.75
Sample after 2 g $\text{NCl}_3$ alone	1.08
Sample after 2 g $\text{NCl}_3$ followed by 0.75 oz $\text{Cl}_2$	0.80
2 g $\text{NCl}_3$ followed by 0.75 oz $\text{Cl}_2$ followed by 1.5 g $\text{NO}_2$	0.69
Sample after 2 g $\text{NCl}_3$ alone	1.07
Sample after 2 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$	0.86
0.75 oz $\text{Cl}_2$ followed by 2 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$	0.69
Sample after 0.75 oz $\text{Cl}_2$ followed by 2 g $\text{NCl}_3$	0.77
0.75 oz $\text{Cl}_2$ followed by 1.5 g $\text{NO}_2$ followed by 2 g $\text{NCl}_3$	0.78
Sample after 0.75 oz $\text{Cl}_2$ followed by 1.5 g $\text{NO}_2$	0.89
1.5 g $\text{NO}_2$ followed by 0.75 oz $\text{Cl}_2$ followed by 2 g $\text{NCl}_3$	0.86
Sample after 1.5 g $\text{NO}_2$ followed by 0.75 oz $\text{Cl}_2$	1.01
1.5 g $\text{NO}_2$ followed by 2 g $\text{NCl}_3$ followed by 0.75 oz $\text{Cl}_2$	0.86
Sample after 1.5 g $\text{NO}_2$ followed by 2 g $\text{NCl}_3$	0.92
Unbleached flour	2.20

in the application of three different gaseous reagents to flour in sequence, the last two reagents should be applied in the order of application found best from the color removal standpoint in the treatment of flour with these two reagents alone.

In Table III six different sequential applications of 0.75 ounces chlorine, 2 g nitrogen trichloride, and 1.5 g nitrogen peroxide per barrel of flour are shown. The results indicate that the nitrogen peroxide should be applied last and that it makes little difference whether nitrogen trichloride is applied first followed by chlorine or chlorine is applied first followed by the nitrogen trichloride.

It will be noted that the color removal achieved by the third reagent in the examples shown in Tables II and III is relatively small, although



it is significant at the low levels of residual carotene content involved. There is much doubt as to whether the third gas could be justified commercially to secure so little additional color removal.

Examples of the application of one gaseous reagent to flour followed by the simultaneous treatment of the same flour with two different gaseous bleaching agents are shown in Table IV. Results achieved

TABLE IV  
BLEACHING OF PATENT FLOUR WITH A GASEOUS REAGENT FOLLOWED  
BY TWO OTHER DIFFERENT GASEOUS REAGENTS APPLIED  
SIMULTANEOUSLY IN BATCH SCALE EQUIPMENT

Bleaching treatment per bbl flour	Carotene p.p.m.
2 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$ and 0.5 oz $\text{Cl}_2$ —applied simultaneously	0.80
Sample after first reagent above	1.10
2 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$ and 0.75 oz $\text{Cl}_2$ —applied simultaneously	0.75
Sample after first reagent above	1.10
0.8 g $\text{ClO}_2$ followed by 1.5 g $\text{NO}_2$ and 0.5 oz $\text{Cl}_2$ —applied simultaneously	0.82
1.5 g $\text{NO}_2$ followed by 0.5 oz $\text{Cl}_2$ and 0.8 g $\text{ClO}_2$ applied simultaneously	0.96
0.8 g $\text{ClO}_2$ followed by 1.5 g $\text{NO}_2$ and 0.75 oz $\text{Cl}_2$ —applied simultaneously	0.86
1.5 g $\text{NO}_2$ followed by 0.8 g $\text{ClO}_2$ and 0.75 oz $\text{Cl}_2$ —applied simultaneously	0.89
Unbleached flour	2.20

show about the same residual carotene content in the flour as can be realized from the treatment of flour with only two gases applied in the preferred order of application from the color removal standpoint. Consequently, this three gas treatment is not considered to have much promise. Again in Table IV it is illustrated that nitrogen peroxide should not be applied to flour first in multiple bleaching treatments.

TABLE V  
BLEACHING OF PATENT FLOUR BY THE SIMULTANEOUS APPLICATION  
OF TWO GASEOUS REAGENTS FOLLOWED BY A THIRD GASEOUS  
REAGENT IN BATCH SCALE BLEACHING APPARATUS

Bleaching treatment per bbl flour	Carotene p.p.m.
0.8 g $\text{ClO}_2$ and 0.5 oz $\text{Cl}_2$ simultaneously followed by 1.5 g $\text{NO}_2$	0.80
0.8 g $\text{ClO}_2$ and 0.75 oz $\text{Cl}_2$ simultaneously followed by 1.5 g $\text{NO}_2$	0.78
2.0 g $\text{NCl}_3$ and 0.75 oz $\text{Cl}_2$ simultaneously followed by 1.5 g $\text{NO}_2$	0.80
2.5 g $\text{NCl}_3$ and 0.50 oz $\text{Cl}_2$ simultaneously followed by 1.5 g $\text{NO}_2$	0.77
0.75 oz $\text{Cl}_2$ and 1.5 g $\text{NO}_2$ simultaneously followed by 2.0 g $\text{NCl}_3$	0.74
0.75 oz $\text{Cl}_2$ and 1.5 g $\text{NO}_2$ simultaneously followed by 0.8 g $\text{ClO}_2$	0.69
Unbleached flour	2.20

Bleaching of flour with two different gases simultaneously, followed by treatment with a third gaseous reagent, is shown by several examples in Table V. Generally speaking, this method of bleaching with three gaseous reagents is not promising since the color removal achieved does not appear to be substantially greater than that which would be realized by the treatment of the flour with two reagents in their pre-

ferred order of application from the color removal standpoint. It may therefore be concluded that the use of three gaseous bleaching reagents on flour does not seem practical from the color removal standpoint when they are applied in any of the ways previously listed and illustrated. Numerous bakings of these samples and other similar ones bleached with three reagents indicated that no advantage nor any greater flexibility was gained from the baking standpoint in these three gas treatments.

The flexibility and advantages sought but not achieved in three-reagent bleaching were found to be obtainable by bleaching different aliquots of a given flour with two different combinations of two gaseous reagents and then blending the resulting bleached flours. An example of this type of treatment, which is capable of producing quite surprising results, is shown in Table VI. In this case, both portions

TABLE VI  
BLENDING OF TWO PORTIONS OF THE SAME FLOUR BLEACHED WITH  
DIFFERENT GASEOUS REAGENT MULTIPLE BLEACHING COMBINATIONS

Flour sample	Bleaching treatment per bbl flour	Bread characteristics			Carotene p.p.m.
		Crumb color	Crumb grain	Loaf volume	
1	2.5 g $\text{NCl}_3$ plus 0.032 oz benzoyl peroxide	10	10	2,800	0.80
2	1.75 oz $\text{Cl}_2$ followed by 1.5 g $\text{NO}_2$	10 dull white	10	2,670	0.87
3	3.0 g $\text{NCl}_3$ followed by 1.5 g $\text{NO}_2$	10 dull creamy	10	2,655	0.78
4	Blend of 50% sample 2 and 50% sample 3	11	10+	2,795	0.83

of the flour were bleached with nitrogen peroxide, the cheapest and most economical of gaseous reagents. Part of the flour had its baking properties influenced by chlorine, part by nitrogen trichloride, and the effects were supplementary in character as can readily be seen by the baking results of the blended flour. This type of multiple bleaching treatment is cheap and provides great flexibility, resulting in optimum baking characteristics in the bleached flour when applied correctly. It can of course be applied to different flours as well as to two portions of the same flour.

### Summary

In the multiple bleaching of flour with chlorine and hypochlorous acid either order of sequential application of these reagents has been

found to be greatly superior from the color removal standpoint to the simultaneous application of the two gases to the flour.

The multiple bleaching of flour with three gaseous reagents does not seem commercially feasible. It offers no distinct advantages either from the color removal or baking standpoints.

Very efficient bleaching of the color and great flexibility in effects on baking characteristics may be obtained by blending two of the same or different flour streams bleached with two different gaseous reagent multiple bleaching combinations.

Many possible bleaching treatments have been described. To recapitulate the findings in this and the preceding papers of this series, the greatest bleaching efficiency in the application of two gaseous reagents to flour results when the reagent higher up in the following list is applied first, followed by the one lower in the list:

Chlorine  
Hypochlorous acid,  
Chlorine dioxide  
Nitrogen trichloride  
Nitrogen peroxide

No preference in order exists for the combination of chlorine and hypochlorous acid or that of nitrogen trichloride and hypochlorous acid.

#### Acknowledgments

The authors wish to acknowledge the assistance of B. A. McClellan in the baking evaluations of the experimentally bleached flours.

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# FLOUR BLEACHING—EXPERIMENTAL APPARATUS AND PROCEDURES<sup>1</sup>

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The purpose of this paper is to describe the generating and metering equipment, techniques, and methods of analysis used by the writers in the handling of solid and gaseous reagents for flour bleaching experiments. Results obtained with this equipment and these procedures are given in the series of papers on multiple bleaching of flour by Ferrari *et al.* (1945 and 1947).

## Flour Agitation

*Laboratory Apparatus.* A simple means of flour agitation is used for the laboratory batch-scale experiments. This apparatus consists of MacLellan batch mixers, preferably of stainless steel with hollow shafts to facilitate the application of the bleaching gases. The maximum capacity of each unit is about 8 pounds of flour. As little as 2 pounds of flour can be bleached, but it is not advisable to go below 2 pounds. Accuracy increases as the amount of flour bleached increases, so a 4- or 8-pound sample is better than a 2-pound sample. Since these mixers rotate at a slow speed of about 5 rpm, the proportion of flour suspended in the air at any given instant is relatively low in comparison with that produced by high-speed commercial agitators. Consequently, the selected amount of gaseous reagent must be applied slowly in order to produce uniform and optimum results. The total bleaching time for a capacity batch was found to be 20 minutes per application. The MacLellan batch mixers contain a set of 6 well-designed baffles placed properly with respect to the design of the shell and the speed and direction of rotation to achieve excellent mixing action and constantly expose new flour surfaces. For the most accurate results, a mixer of this type with baffles is believed to be necessary.

The gas is conducted from the generator by means of a flexible tube and is admitted to the mixer through the hollow shaft by means of a special Bakelite connection (see Figure 2), the hollow shaft of the mixer being lined with a glass tube to prevent corrosion of the metal and subsequent flour contamination. The opposite shaft is hollow with an opening which is partially filled with glass wool or cotton to

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prevent pressure build-up. Perfectly uniform color removal throughout the batch is obtained with this type of agitation. The results are easily replicated.

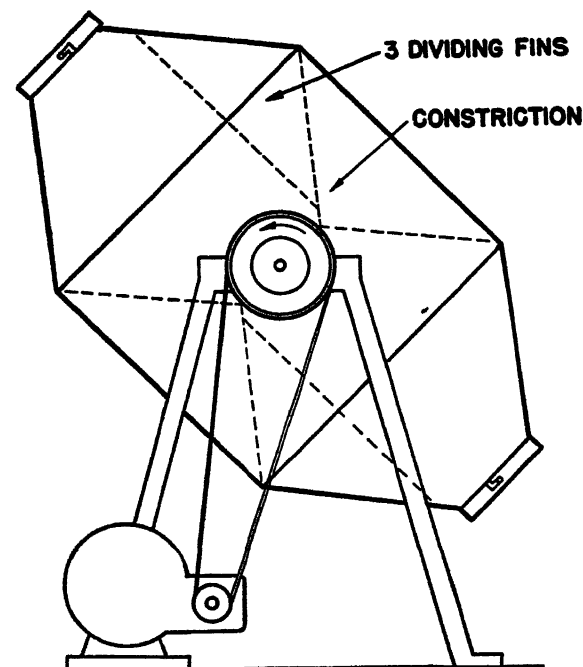


Fig. 1. Batch bleaching agitator—MacLellan mixer with hollow shafts.

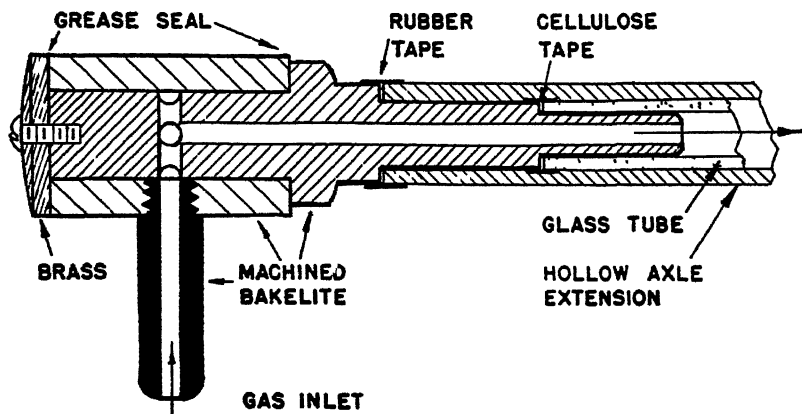


Fig. 2. Diagram showing construction features of bakelite connection for admitting gaseous bleaching reagents to laboratory batch mixer.

Larger mixers of this same type have been found effective in bleaching greater quantities of flour experimentally.

*Pilot-Scale Equipment.* The pilot-scale agitator consists of a chamber in which paddles rotate at high speed to simulate the action of a commercial agitator. This machine is contained in equipment capable of handling a continuous flour stream of 1 to 5 barrels per hour.

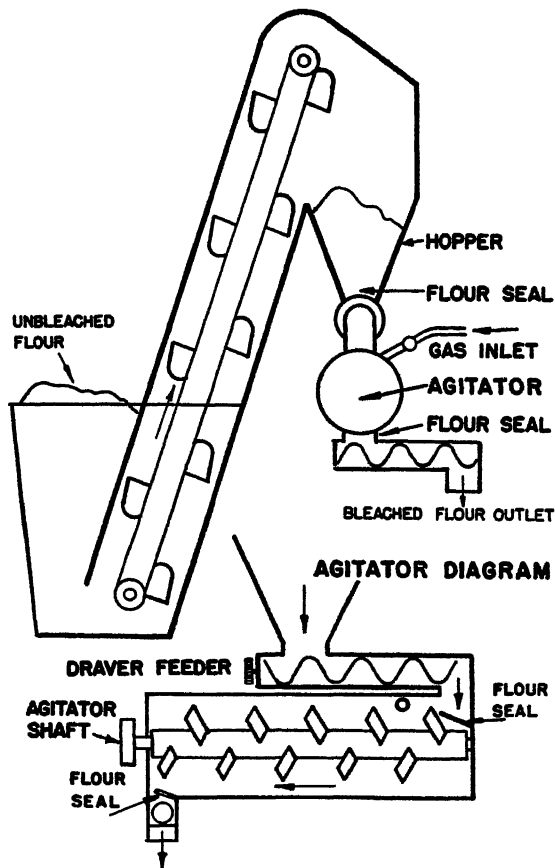


Fig. 3. Diagram of pilot-scale flour-handling and agitating equipment.

A Draver screw conveyor delivers a uniform flour stream to the agitator which has a flour seal at the flour inlet and exit points. Any desired bleaching gas or combination of gases can be admitted to the agitator chamber at a point directly below the flour inlet. Four gas inlets fitted with shut-off valves are provided to allow maximum flexibility. For multiple bleaching experiments with this apparatus, the flour must be recycled for each bleach which is to be applied separately or in sequence. Since the bleaching action is continuous, gases generated by commercial equipment as well as by experimental

apparatus may be used. The desired amount of gas is metered to the agitator and any excess is by-passed into an exhaust hood.

*Commercial Agitators.* Commercial agitators are essentially the same as the pilot equipment described except for their greater size and capacity. For multiple bleaching work, two or more agitators are connected in series with flour seals at the flour inlet and exit points of each unit. The gases are introduced at a point just below the flour inlets.

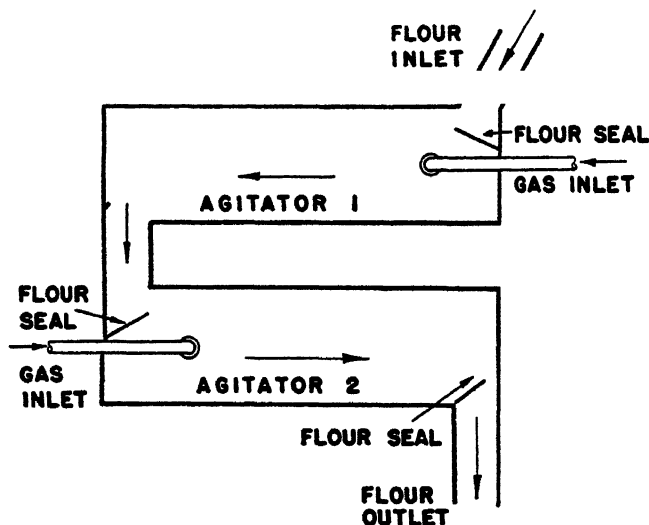


Fig 4 Diagram of multiple bleaching agitator system.

### Bleaching with Solid Reagents

Solid bleaching reagents, such as those containing benzoyl peroxide or fatty acid peroxides, are added before, during, or after the treatment with gaseous reagents. They are added as a single treatment in batch or pilot-scale experiments in MacLellan batch mixers of various sizes. At least 15 minutes is allowed for the blending of the reagent and the flour. From 10 to 36 hours is needed for the full bleaching effect of solid reagents to take place; so the color analysis is not made on the treated flour sample until sufficient time has elapsed.

### Bleaching Gas Generators and Procedures

Apparatus utilized for the generation and metering of various bleaching gases provides for small-scale production of these gases with sufficiently flexible and accurate control to perform multiple bleaching and other bleaching experiments on flour. In a few instances indicated

below, available commercial equipment is used where feasible for batch and pilot-scale experiments.

Accurate control of each gas is accomplished by metering or weighing one constituent of the reaction mixture producing the gas. The gas is stripped from the reaction zone and conducted to the bleaching agitator by the use of metered air. The efficiencies of the reactions are determined by analysis of the effluent gases.

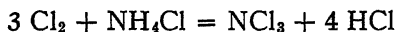
*I. Chlorine Dioxide.* Equipment or apparatus and procedures for the carefully controlled generation and metering of chlorine dioxide for flour bleaching on a laboratory, pilot, or commercial scale have been fully described in recent publications by Hutchinson and Derby (1945) and Woodward, Petroe, and Vincent (1944). In each case the method used is the gaseous chlorine, solid chlorite process of Hutchinson and Mecham (1943). Procedures for the analysis of the effluent gaseous mixtures for chlorine dioxide are given in the first two references cited above.

*II. Nitrogen Trichloride.* A. LABORATORY SCALE. Small-scale equipment for the generation of nitrogen trichloride consists of a gas-measuring burette and reaction chamber with proper connections for stripping the gas with metered air. When the gas is prepared in a reaction chamber and this unit amount of gas is stripped from the chamber and conducted to the flour, accurate metering of the air is not necessary for the bleaching process. However, with the equipment used, there was found to be an optimum rate of stripping air of about 300 ml per minute.

A diagram of the apparatus is shown in Figure 5. The procedure for generating nitrogen trichloride with this particular equipment is as follows: The chlorine line is filled with pure gas up to point *B* and the desired quantity is metered into the burette, displacing a saturated salt solution. The reaction tube which is first filled with a solution containing about 6 g of ammonium chloride per 200 ml of water is placed under vacuum sufficiently strong to draw all the metered chlorine into the tube through the porous diffusing cylinder. The vacuum is completely released by allowing air to enter the system at point *G* in order to flush the remaining chlorine into the reaction vessel. The reaction tube is then shaken to insure that all the chlorine is reacted, and the resulting nitrogen trichloride is removed from the tube by forcing the stripping air through the porous cylinder. The stripping time allowed for a 200 ml reaction tube was about 20 minutes, although most of the nitrogen trichloride is carried to the agitator in the first few minutes.



According to Ferrari (1928), nitrogen trichloride is produced according to the following over-all equation:



The volume of chlorine needed for the bleaching treatment was calculated from this equation, proper corrections being made for pressure and temperature conditions. An excess of ammonium chlo-

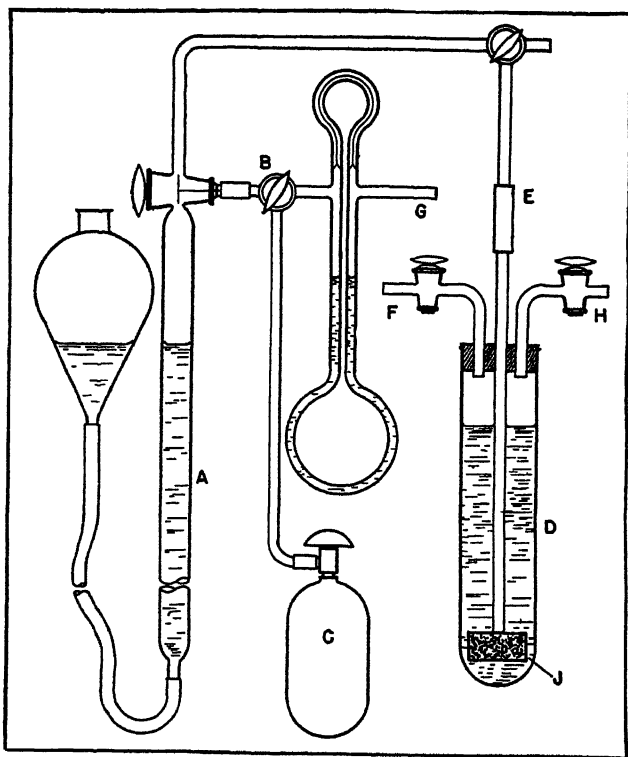


Fig. 5. Laboratory scale apparatus for the production of nitrogen trichloride. A. Gas burette. B. 3-way stopcock. C. Chlorine cylinder. D. Reaction tube. E. Flexible section. F. Vacuum connection. G. Air inlet. H. To agitator. J. Porous cylinder.

ride was always present, so the control of the treatment was on the chlorine input.

Bleaching results with this generator and the MacLellan batch mixer were found to be easily replicated, two samples of the same flour treated with the same dosage of  $\text{NCl}_3$  usually varying less than 0.03 ppm in carotene content.

Ferrari, Hutchinson, and Mecham (1945) found that the efficiency of bleaching with this laboratory-scale generator and the MacLellan batch mixer is greater than in the nitrogen trichloride pilot-plant

equipment which in turn is more efficient than the commercial-scale equipment.

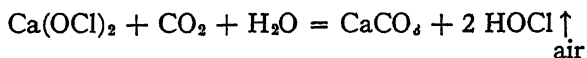
As a check on the proper functioning of the apparatus and application of the nitrogen trichloride gas to the flour, carotene analyses are usually made on the flour both before and after the bleaching treatment. Through experience in bleaching certain grades of flour, this provides a good check on the probable accuracy of the treatment; much better than can be obtained by the "Slick" or Pekar test. The carotene value is, of course, valuable for other reasons.

Flour color is expressed as "carotene" and is measured by the procedure described by Binnington, Hutchinson, and Ferrari (1941). Briefly stated, flour pigments are extracted with water-saturated butyl alcohol and the extract read in an Evelyn photoelectric colorimeter using a 440-m $\mu$  filter. The carotene results are converted to the more customary naphtha-alcohol carotene basis by using the following equation established by Binnington, Sibbitt, and Geddes (1938): Naphtha-alcohol "carotene" = 0.8075 (Butyl alcohol "carotene" - 0.14).

**B. PILOT SCALE.** Commercial-scale Agene equipment supplied by the Wallace and Tiernan Company is used with the pilot-scale conveyor and agitator equipment already described.

This commercial equipment may be used also for small-scale batch bleaching by maintaining a predetermined level of nitrogen trichloride generation and conducting a part of the gas through a flow meter into the flour blenders or agitators for a definite period of time. The treatment is calculated from the following factors: (1) the proportion of the total effluent diluted gas from the generator that is conducted to the flour, (2) the nitrogen trichloride level in the generated gas, (3) the time the gas is applied to the flour, and (4) the amount of flour treated. The excess gas is conducted into an exhaust hood or absorbed in caustic alkali or sodium sulfite.

**III. Hypochlorous Acid.** The following equation represents the probable course of the reaction that is utilized in the production of hypochlorous acid:



Hypochlorous acid as a gas is used in quantities up to 25 g per barrel to bleach small unit amounts of flour (2 to 8 pounds) in the MacLellan blenders. This reagent may be generated according to the above equation by reacting a calculated amount of powdered calcium hypochlorite of known available chlorine content in a closed chamber with an excess of carbon dioxide in the presence of moisture, the hypochlorous acid being stripped from the reaction chamber by air.

Hypochlorous acid may be prepared by first weighing a small calculated amount of calcium hypochlorite of about 60-70% available chlorine content into a 1-liter Erlenmeyer flask. (For a dosage of 25 g hypochlorous acid per barrel on 4 pounds of flour, 1.23 g of calcium hypochlorite at 61.5% available chlorine content is required.) About a gram of moisture is added to the flask in the form of steam in such a

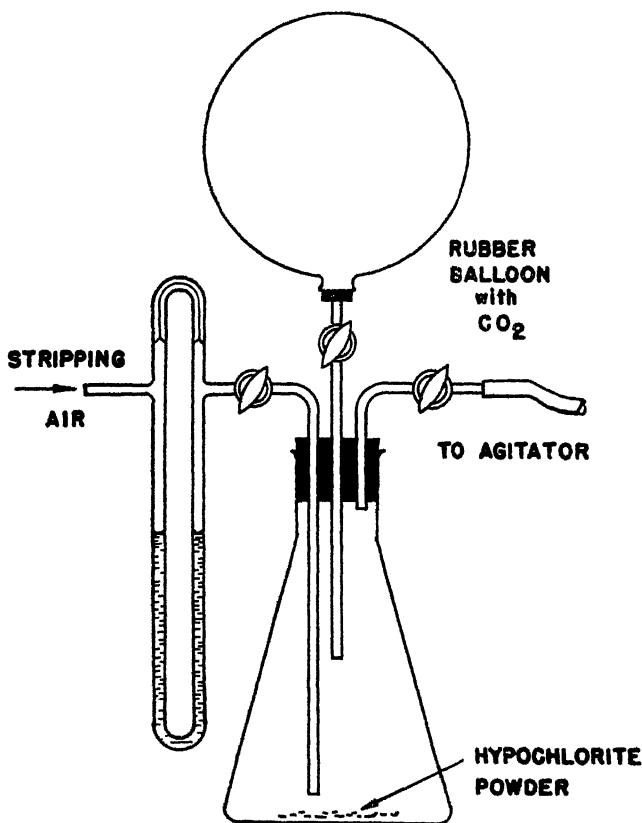


Fig. 6. Apparatus for the small-scale generation of hypochlorous acid.

manner that no large droplets form at any one point in the flask. The stopper containing the stopcock and tubing is inserted immediately and the flask partially evacuated with a water pump. The vacuum is released with carbon dioxide from a balloon. At room temperatures this method has an optimum reaction time of about  $1\frac{1}{2}$  to 2 hours in the dark, efficiencies of 93 and 89%, respectively, having been found for these reaction times. Optimum yield of hypochlorous acid depends both on the time of reaction and the amount of moisture present in the flask. The amount of moisture found necessary is that which

will be sufficient for the needs of the reaction in contact with the calcium hypochlorite as liquid or vapor and which is present in small enough excess so that the excess will be completely evaporated by an air flow of about 300 ml per minute for 20 minutes, which is the amount of air used for stripping the hypochlorous acid from the reaction vessel. If more moisture were present, the stripping time would have to be increased until the flask is dry; otherwise, some of the hypochlorous acid remains dissolved in the moisture in the flask. If too little moisture is present, the reaction does not reach completion, and the percentage yield will drop off considerably.

**ANALYTICAL DATA:** The analysis of the bleaching powder is determined by titrating with standard thiosulfate the iodine liberated from potassium iodide added to a dilute solution of the calcium hypochlorite acidified with dilute sulfuric acid.

The percentage yield of hypochlorous acid in the process described is determined in two steps. First, hypochlorous acid is generated in a flask as described, and after allowing  $1\frac{1}{2}$  hours to complete the reaction, the flask is swept with air at 300 ml per minute for about 20 minutes, at which time no noticeable moisture remains. The residue is then analyzed by the method indicated above for the bleaching powder. It has been found that over 99% of the available chlorine reacts and is displaced from the flask. Thus it becomes possible to prepare hypochlorous acid in a flask as described and conduct an analysis of its oxidizing power or yield by adding 50 ml of 1 *N* sodium hydroxide solution to the flask by means of a pipette and a rubber connector and shaking the flask in order to dissolve or react all of the hypochlorous acid. Since the reaction to produce the hypochlorous acid creates a slight vacuum in the flask, the sodium hydroxide solution can be readily admitted.

After allowing a short time for the sodium hydroxide to react with the hypochlorous acid, potassium iodide is added to the solution, after which it is neutralized to a phenolphthalein end point and made acid with 10 ml of glacial acetic acid. From a standard thio titration, the amount of hypochlorous acid produced can be calculated.

**IV. Nitrogen Dioxide.** In order to conduct laboratory flour-bleaching experiments with nitrogen dioxide gas, a commercial Alsop generator complete with standard electrical equipment and blower for supplying a flow of air to the flaming arc dome is assembled according to the diagram in Figure 7.

A 5-gallon metal container equipped with inlet and outlet pipes is used in the air line following the Roots Connorsville blower as an expansion chamber in order that the blower pulsations will not be transmitted to the flowmeters and arc discharge unit. The total air

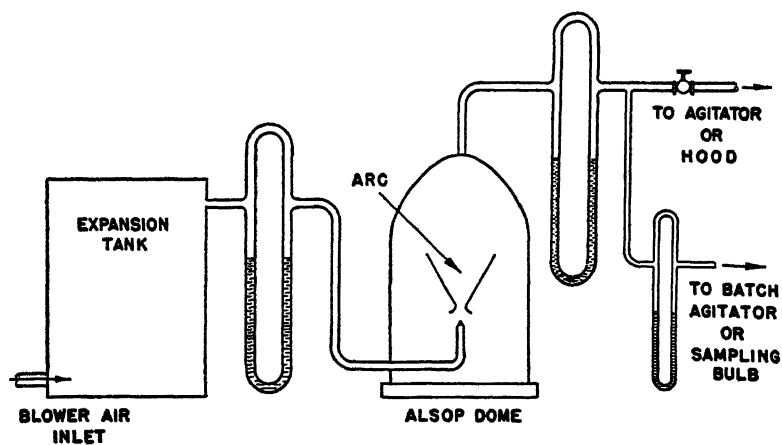


Fig. 7. Diagram of Alsop nitrogen dioxide generator with accessories for laboratory bleaching experiments.

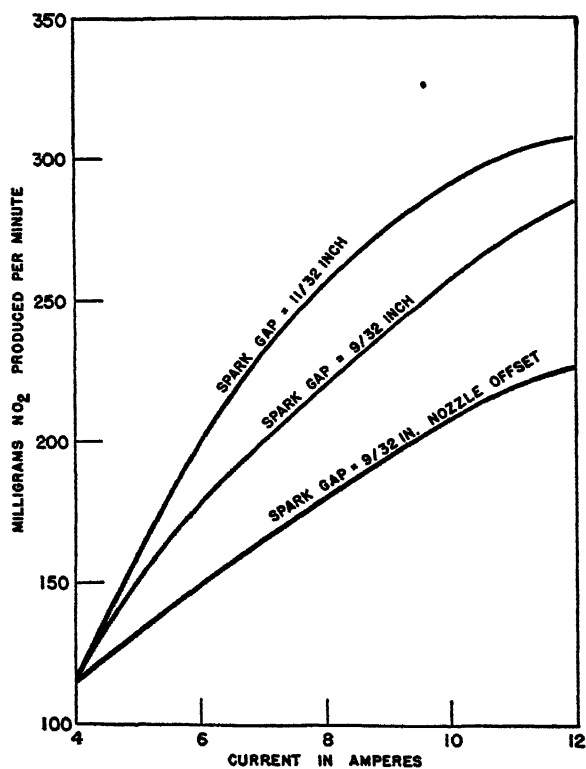


Fig. 8. Performance of test Alsop machine with constant air flow at 0.36 liters per second and with various spark gap adjustments.

flow is measured by the differential flow meters on each side of the Alsop dome. As the dome is heated by the arc discharge, the air volume becomes greater at the dome and the downstream flowmeter indicates a greater gas flow. However, the length of the system with subsequent cooling of the gas tends to cancel this effect, and it is not considered in the analytical calculations.

Before any bleaching work was done with the Alsop apparatus, it was thought desirable to calibrate the output of the machine. The

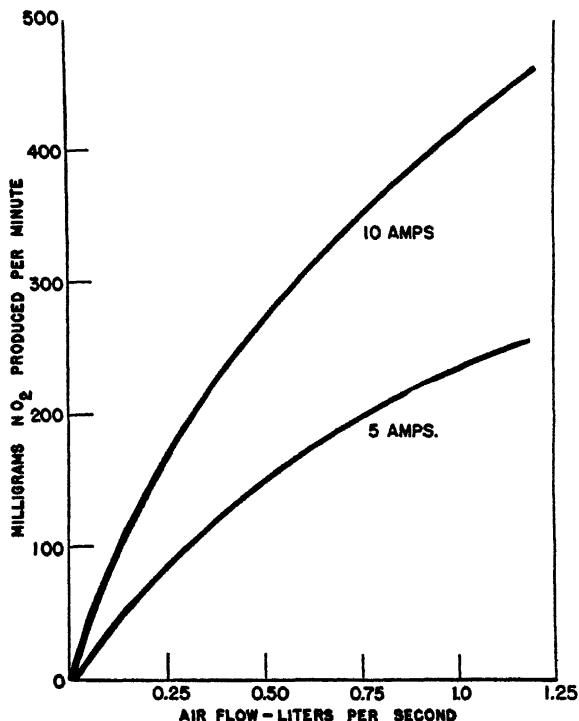


Fig. 9. Effect of air flow and amperage on the nitrogen dioxide production of the Alsop machine.

effluent gas was analyzed while the generator was operating at various measured air flows and at various levels of current input to the arc. The electrode spacing was also varied, as was the position of the electrodes over the air nozzle.

During the preliminary calibration, it soon became apparent that although the amount of nitrogen dioxide produced is controlled primarily by air flow and amperage, the other factors also have an important effect on the nitrogen dioxide output. The nitrogen dioxide production, of course, increases with current. Analysis of the effluent gases showed the extent to which the change in the spacing between the

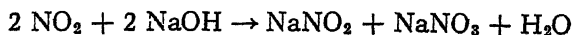
electrodes affects the nitrogen dioxide production. Also demonstrated was the fact that nitrogen dioxide production was lowered considerably if the electrodes were not in optimum alignment with the air nozzle, an adjustment that appears to be very critical.

The nitrogen dioxide productions with constant air flow for electrode gap settings at 11/32 inch and 9/32 inch and also for the gap at 9/32 inch with the electrodes offset slightly from the air nozzle are shown in Figure 8. It is seen that the nitrogen oxide output increases with increased space between the electrodes. It is greatest when the spark gap is centered over the air nozzle.

The effects of variations in air flow on nitrogen peroxide output are shown in Figure 9. For each amperage level investigated the production of nitrogen oxides is increased markedly as the air flow is increased over the range permitted by the equipment.

During the calibration of the Alsop generator, the main gas line is exhausted to a hood, and a small amount of gas (300 ml per minute) is diverted into a gas sampling bulb of known volume (Figure 10). Because of partial pressure considerations, the total air flow is held at about 0.36 liters per second or 21.6 liters per minute as measured by the differential flow meters. The gas valve in Figure 7 has to be closed somewhat in order to divert the required flow of gas to the sampling bulb. The back pressure caused by this action is measurable, although very slight. The flowmeters are calibrated under practically the same back pressure; however, such refinements are not considered necessary and in terms of bleaching effect usually represent an insignificant flour color removal value.

When the Alsop machine is considered to have reached equilibrium using any desired setting from 4 to 12 amperes of current to the transformer, the bulb sample is taken. The 50 ml bulb on the sampling device is blown out with air and filled with 50 ml of 1 *N* sodium hydroxide solution. A stopper is placed in the small bulb, and the sodium hydroxide solution is allowed to flow down into the sample chamber by opening the connecting stopcock and adjusting the three-way stopcock in such a manner that the gas may circulate throughout the whole closed apparatus. The absorption or reaction time allowed varies from 3 hours to overnight. Analytical procedure: the reaction which takes place in the sampling bulb between nitrogen dioxide and sodium hydroxide may be shown by the equation:



Thus the nitrite formed represents one-half of the nitrogen dioxide in the sampling bulb. The nitrite formed is determined quantitatively

by the colorimetric method of Griess and Ilsoyay, as described by the Association of Official Agricultural Chemists (1935).

The standard nitrite solution is prepared by dissolving 0.1097 g silver nitrite in 20 ml of hot water, precipitating the silver with 0.10 g of sodium chloride, and making up to 1 liter. When 10.00 ml of this

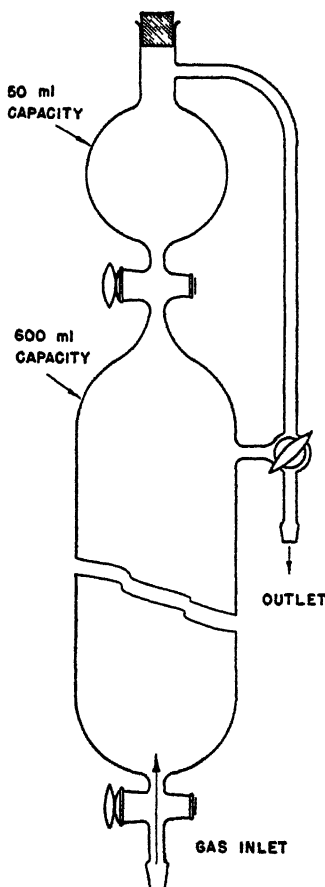


Fig. 10. Gas sampling bulb.

solution is diluted to 1000 ml, a standard solution is obtained which contains  $3.28 \times 10^{-4}$  mg of nitrite ion per ml. The solutions of sulfanilic acid and  $\alpha$ -naphthylamine hydrochloride are prepared as directed in the above citation. The reference color standards are made up daily containing from 3.00 ml to 6.00 ml of standard nitrite solution by 0.25 ml increments diluted to 100 ml in matched Nessler tubes. Small differences in nitrite concentration are most easily distinguished in this range.



Since the gas sample is absorbed in alkali, the effect of the addition of alkali to the color standards was studied in connection with the rate of color development and the final color obtained. The effect of the sodium chloride produced by neutralizing the sodium hydroxide with hydrochloric acid was also determined.

The results of these tests indicated that an addition of more than 0.5 milliequivalents of sodium hydroxide had a definite retarding effect on the rate of color development and the final color obtained. It was also noted that this effect could not be controlled by neutralization with hydrochloric acid. The dilutions of the absorbed nitrogen dioxide solutions are manipulated in such a way as to insure that only 0.025 to 0.10 milliequivalents of sodium hydroxide are present when the color-forming reagents are added.

After the gas sample has reacted with the 50 ml of 1 *N* sodium hydroxide solution, a 5.00 ml aliquot is withdrawn and diluted to 1 liter. From this diluted aliquot, 5.00 ml to 20.00 ml, according to the strength of the sample, are transferred for the colorimetric determination to a 100 ml Nessler tube and 2.0 ml each of the sulfanilic acid and the  $\alpha$ -naphthylamine hydrochloride solutions are added and the volume made up to 100 ml with distilled water. After mixing and standing at least one hour, stoppered, the Nessler tube containing the diluted sample which has achieved a color proportional to the concentration of the nitrite therein is matched with one of the tubes containing the standard nitrite solution, and the amount of nitrogen dioxide produced per minute is calculated by the following equation:

$$\text{Mg NO}_2/\text{min} = \frac{(\text{ml N NaOH}) \left( \frac{\text{ml dilution}}{\text{ml diluted sample to Nessler tube}} \right)}{\text{ml aliquot}} \frac{(\text{ml standard}) (3.28 \times 10^{-4}) \left( \frac{1000 \text{ ml}}{\text{ml vol. of sample}} \right)}{(\text{air flow, liters/min})} \quad (2)$$

Sample calculation: the Alsop generator is operated at 12 amps. and 21.6 liters per minute of air flow. The volume of the sample is 618 ml. After reacting the gas with 50 ml of 1 *N* sodium hydroxide solution, a 5.0 ml aliquot is diluted to 1 liter. From this diluted solution a 4 ml aliquot in the Nessler tube with the color-forming reagents and water matches the tube containing 5.25 ml of the standard solution. The milligrams of nitrogen dioxide produced per minute in the effluent gas is calculated by substituting in the above equation:

$$\text{Mg NO}_2/\text{min} = \frac{50}{5} \times \frac{1000}{4} \times 5.25 \times 3.28 \times 10^{-4} \times \frac{1000}{618} \times 21.6 \times 2 = 300$$

The various sample dilutions may be manipulated to suit particular requirements with the minimum dilution dependent on the concentration of alkali in contact with the color-forming reagents as previously mentioned.

### Batch-Scale Bleaching with Nitrogen Peroxide

After the generator is calibrated for one air flow and a range of ampere settings, it can be used for accurate small-scale batch bleaching. The assembly is practically the same as for the calibration, with most of the gas being exhausted into a hood. A measured or metered part of the total known flow of gas is admitted to the small MacLellan mixer in which a weighed amount of flour is being agitated. This metered amount of gas is introduced to the flour for the length of time shown by the calibration data to be required to produce the desired treatment of the flour.

Convenient time and flow for small dosages may be worked out at 10 amperes of current so that the generator is working near capacity. Then, in the event large-scale bleaching is desired, the apparatus need not be changed, the dosage being regulated by the amount of gas introduced to the flour.

*V. Chlorine.* A. LABORATORY SCALE. The test bleaching of flour with chlorine gas is easily and accurately accomplished by use of the laboratory nitrogen trichloride apparatus previously described. The apparatus is modified to the extent of exchanging the reaction tube for a 250 ml wide-mouth bottle fitted with two long inlet tubes and one short exit tube as shown in Figure 11. This bottle *D* acts as a gas-mixing chamber which allows metered chlorine from *A* through *E* to be diluted with air conducted from *G* through *F* (three-way stopcock *B* being closed), which sweeps it to the blender and flour.

Considering the nature of the flour agitation in a MacLellan blender, it is recommended that the chlorine be admitted to the gas and air mixing chamber at a rate not to exceed 25 ml per minute with the air flow at about 300 ml per minute. This procedure will produce a uniform bleach throughout the sample. Uniformity of results is not so easily obtained if the rate of chlorine application is too great.

After all of the chlorine is forced from meter *A* by the leveling bulb, line *E* is opened to the inlet air by partially closing pinch clamp *F* and opening stopcock *B* to this air flow. This operation insures that all of the metered chlorine in the apparatus is conducted to the flour.

Since no reaction takes place, no analytical control is necessary for this apparatus.

B. PILOT SCALE. The pilot-scale bleaching of a flour stream with chlorine is done with commercial scale equipment which is adjustable

for continuous flow on small streams. For a very small continuous gas flow, the output from the commercial apparatus may be proportioned by use of differential manometers. A metered part of the total known flow of gas may be admitted to the flour agitator.

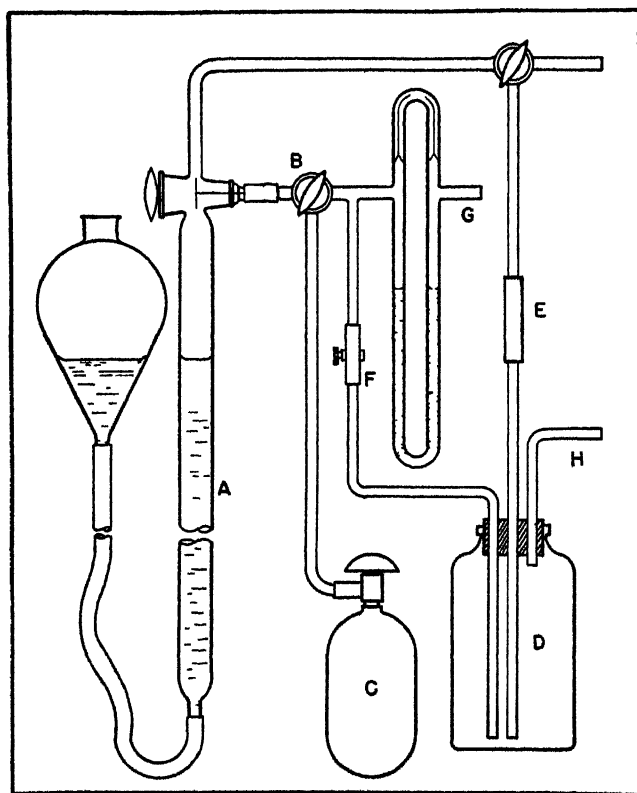


Fig. 11. Chlorine metering device for small-scale bleaching. A. Gas burette. B. 3 way stop cock. C. Chlorine cylinder. D. Gas mixing chamber. E. Flexible connection. F. Screw clamp. G. Air inlet. H. Gas outlet to flour agitator.

### Summary

Apparatus and procedures for the experimental bleaching of flour with nitrogen trichloride, hypochlorous acid, nitrogen dioxide, and chlorine are described in detail and those using chlorine dioxide outlined briefly.

Agitating equipment suitable for flour bleaching on a laboratory, pilot, or commercial scale is described. The generation and metering of nitrogen trichloride, chlorine, and nitrogen dioxide for laboratory, pilot, and commercial practice are discussed. A method is described for the laboratory-scale production of hypochlorous acid.

### Acknowledgments

The assistance of Dale K. Mecham in developing the analytical procedure for Alsop gas analysis is gratefully acknowledged.

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# SOME FACTORS INFLUENCING THE PROTEIN, CYSTINE, AND METHIONINE CONTENT OF DRY PEAS<sup>1</sup>

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Cystine and methionine have been shown to be the limiting amino acids of the proteins of dry peas for nutrition (Johns and Finks, 1921; Woods, Beeson, and Bolin, 1943; Peterson, Lampman, Bolin, and Stamberg, 1944). Considerable variation in both the cystine and methionine contents of dry peas was reported by Evans (1945) for samples of peas obtained on the open market and differing in variety. They were probably grown in different years and under different climatic conditions.

Different varieties of soybeans have different nitrogen and cystine contents (Hamilton and Nakamura, 1940). Different varieties of wheats and wheats receiving different treatments have been shown to differ in sulfur and nitrogen (Greaves and Bracken, 1937), and in cystine content (Gubler and Greaves, 1942). The sulfur content of alfalfa was shown by Evans and Greaves (1937) to vary with variety, crop, and fertility of the soil.

It appeared desirable to study the influence of certain factors on the protein, cystine, and methionine in dry peas to see if the protein and sulfur of dry peas can be raised by some method of management or by use of particular varieties.

## Materials and Methods

Alaska peas were used to study the influence of fertilization on the protein, cystine, and methionine in dry peas. The experimental plots were located in the Palouse area of eastern Washington. Six fertilizer treatments were used: sodium nitrate (100 pounds per acre), ammonium sulfate (100 pounds per acre), sulfur (20 pounds per acre), gypsum at the rate of 100 pounds per acre, gypsum at the rate of 200

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pounds per acre, and sodium nitrate and gypsum (each 100 pounds per acre). Each treatment was grown in each of three locations. One series was grown on the north slope, one on the south slope, and one on the hilltop.

The two varieties of peas produced in the Palouse area which are most used for dry peas are Alaska, and First and Best. These two varieties along with White Canada, a late-maturing pea, were studied to see if variety had any influence on the protein, cystine, and methionine content of dry peas. Each variety was replicated four times.

Alaska peas were used to determine the influence of date of harvest, or maturity, on the protein, cystine, and methionine in dry peas. Six plots were used, all planted at the same time. Duplicate plots were harvested on July 17, 1944, when the vines were beginning to show signs of maturity by the presence of some dead leaves although plants and pods were still definitely green. Two plots were harvested on July 22, 1944, when the vines were thought to be intermediate in maturity between the July 17 harvest and the mature stage. The remaining two plots were harvested on August 2, 1944, when the vines were completely dry. The peas were mature about July 29.

The samples were ground in a Wiley mill and well mixed soon after harvesting. The ground samples were then placed in bottles and left sealed till analyzed. All analyses were completed within a year after harvesting. Protein was determined by the Kjeldahl-Gunning-Arnold Method (A.O.A.C., 1945). Total sulfur was determined as described by Evans and St. John (1944), organic sulfur as described by Evans and Greaves (1937), and cystine and methionine by the differential oxidation procedure (Evans, 1945). The question of the specificity of this procedure for cystine and methionine might be raised when used with some materials containing other sulfur compounds than cystine, cysteine, methionine, and sulfate. Evans (1945) showed a close agreement between the differential oxidation procedure for methionine in soybean oil meal and cottonseed meal and the procedure of McCarthy and Sullivan (1941) when corrections were made for losses during acid hydrolysis. A similar study with six samples of dry peas gave an average value of 1.07% methionine in the pea protein by the McCarthy and Sullivan (1941) method and one of 1.03% methionine by the differential oxidation procedure. Differences between the two methods for individual samples were less than differences between different determinations on the same samples by the McCarthy and Sullivan procedure. No comparison could be made between the differential oxidation procedure for cystine and colorimetric methods, because no good method is available for hydrolyzing peas for cystine determination, owing to the large loss of cystine on hydrolysis of sub-

stances such as dry peas (Lugg, 1933). Isolation of the whole protein involves use of alkaline solutions which cause destruction of cystine. Because of this the differential oxidation procedure offers the best method available for the determination of cystine in dry peas.

The results were analyzed statistically by the method of variance (Snedecor, 1937). The variance for error represents variation between replicate plots, and not variation in chemical determinations. All chemical determinations were made in duplicate and the average values used.

### Results and Discussion

*Fertilizers.* The addition of sulfur-containing fertilizers to the soil increased the percent of total sulfur in the dry peas grown thereon, but decreased the protein in these peas (Table I). This held true when

TABLE I  
INFLUENCE OF FERTILIZER TREATMENTS ON PROTEIN CONTENT AND  
SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*)<sup>1</sup>

Treatment	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	lbs/acre	%	%	%	%	%	%
Check (East)	1540	23.8	0.17	0.14	0.57	1.3	1.0
Sodium nitrate	1470	23.6	0.17	0.13	0.54	1.3	0.9
Sodium nitrate and gypsum	1550	22.9	0.21	0.14	0.58	1.6	0.7
Gypsum (low)	1500	22.1	0.22	0.14	0.63	1.8	0.7
Gypsum (high)	1450	21.1	0.22	0.14	0.65	1.8	0.8
Ammonium sulfate	1420	21.9	0.22	0.14	0.63	1.7	0.8
Sulfur	1600	23.5	0.20	0.14	0.56	1.5	0.8
Check (center)	1560	23.0	0.17	0.13	0.55	1.5	0.7
L. S. D. <sup>2</sup>		1.0	0.02	0.01	0.05	0.2	0.3
F value <sup>3</sup>		8.49	16.26	3.50	5.57	14.00	9.76

<sup>1</sup> These values are averages for the three locations. The peas were harvested when mature.

<sup>2</sup> L. S. D. indicates the least difference required for significance at the 5% level.

<sup>3</sup> F values required for significance at the 5% and 1% level are 2.80 and 4.40.

sodium nitrate was applied with gypsum and when ammonium sulfate was applied, as well as when gypsum was used alone. There were practically no differences in organic sulfur content of the whole peas, but the protein of the peas fertilized with ammonium sulfate or gypsum alone contained significantly more sulfur than did the protein of the others. Cystine values in the protein were influenced in the same direction as the sulfur, but the methionine values were not significantly different. None of the differences found were large, and although certain of them were significant statistically, the importance of some of the differences from a nutritional standpoint is questionable.

That climatic conditions may have a significant influence on the composition of dry peas is evident from Table II which presents the average values for dry Alaska peas grown on the south slope, the north slope, and on the hilltop of one of the Palouse hills. Such slopes are common in the hilly Palouse country. The peas grown on the south

TABLE II  
INFLUENCE OF LOCATION ON THE HILL ON PROTEIN CONTENT AND  
SULFUR DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*)<sup>1</sup>

Location	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methi- onine
	lbs/acre	%	%	%	%	%	%
South slope	1580	23.9	0.22	0.11	0.59	1.5	0.9
Hilltop	1160	21.6	0.19	0.13	0.59	1.6	0.7
North slope	1450	22.8	0.19	0.14	0.59	1.6	0.8
L. S. D.	-	0.6	0.01	0.01	0.03	0.1	0.2
F value <sup>2</sup>	-	34.03	17.29	19.25	0.32	2.92	2.39

<sup>1</sup> Eight plots each receiving a different fertilization treatment were harvested from each location. The peas were harvested when mature.

<sup>2</sup> F values required for significance at the 5% and 1% levels are 3.74 and 6.51.

slope were richer in protein and sulfur than those grown on the north slope or the hilltop, although the differences were quite small and their importance from a nutritive point of view might be of little importance. No significant differences in the cystine, methionine, or sulfur in the pea proteins were observed.

TABLE III  
INFLUENCE OF HARVESTING DATES ON PROTEIN CONTENT AND SULFUR  
DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*)<sup>1</sup>

Harvesting date	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methi- onine
	lbs/acre	%	%	%	%	%	%
July 17, 1944	1110	26.4	0.23	0.15	0.58	1.3	1.1
July 22, 1944	1338	26.1	0.23	0.15	0.58	1.2	1.2
Aug. 2, 1944	1254	25.8	0.24	0.16	0.61	1.3	1.3
L. S. D.	-	0.9	0.02	0.01	0.01	0.1	0.3
F value <sup>2</sup>	-	2.14	1.50	-	42.90	3.00	1.38

<sup>1</sup> Average values for two quadrates. No fertilizer was applied, but this field had been previously in alfalfa which was plowed under in 1943. The peas in this field were considered to be mature July 29, 1944.

<sup>2</sup> F values required for significance at the 5% and 1% levels are 9.55 and 30.81.

**Maturity.** Although the protein from the peas harvested at the latest date, August 2, appeared to contain more sulfur than peas harvested earlier, the differences were small and no other statistically



significant differences were observed (Table III). None of the differences would appear to be of sufficient magnitude to be important from a nutritive standpoint.

*Variety.* White Canada peas contained higher levels of protein, total and organic sulfur than did Alaska, or First and Best peas (Table IV). However, the proteins of these three varieties appeared

TABLE IV  
INFLUENCE OF VARIETY ON PROTEIN CONTENT AND SULFUR  
DISTRIBUTION OF DRIED ALASKA PEAS (*Pisum sativum*)<sup>1</sup>

Variety	Yield	Dry matter basis			Protein basis		
		Protein	Total S	Organic S	Organic S	Cystine	Methionine
	<i>lbs/acre</i>	%	%	%	%	%	%
Alaska	726	22.1	0.20	0.16	0.70	1.9	1.0
First and Best	930	22.1	0.21	0.17	0.75	2.0	1.0
White Canada	648	23.6	0.22	0.18	0.74	2.0	1.0
L. S. D.	—	1.0	0.01	0.01	0.03	0.1	0.1
F value <sup>2</sup>	—	8.81	57.00	24.00	6.97	3.75	0.00

<sup>1</sup> Average values for four replications. No fertilizer was applied. The peas were harvested as soon as they were considered to be ripe.

<sup>2</sup> F values required for significance at 5% and 1% levels are 5.14 and 10.92.

to be similar in cystine and methionine contents. It appears from these results that the differences in cystine and methionine levels in the protein of dry peas reported by Evans (1945) were not due to differences in variety, but to some other factor. Further work with additional varieties of peas might show differences comparable to those reported for organic sulfur in wheat (Greaves and Bracken, 1937), cystine in wheat (Csonka, 1937; Gubler and Greaves, 1942), cystine in corn (Doty, Bergdoll, Nash, and Brunson, 1946), and cystine in soybeans (Hamilton and Nakamura, 1940).

Evans (1945) determined the percentage of cystine and methionine in five dry pea samples. Cystine values ranged from 1.4 to 0.9% of the protein and methionine values ranged from 1.6 to 0.8%. Similar variations are reported in the present paper with cystine values of 2.0 to 1.2% of the protein and methionine values of 1.3 to 0.7%. Taken together, cystine values from 2.0 to 0.9% and methionine values between 1.6 and 0.7% were obtained. Although fertilization with gypsum increased the cystine from 1.3 to 1.8% of the protein, no fertilization or soil management factors were observed which would explain the wide differences observed between the different samples referred to above. These must be left for further investigation, as must the reason why the ratio between cystine and methionine varied between 1:1 and 2:1.

Also of interest are the differences in protein content, unexplained by the data presented, of some of the samples of peas. The observation of Wakeham (1943) that crops grown under "poor" conditions may contain more nitrogen and more minerals than crops grown under "good" conditions may be of importance in this regard. Yield data presented for the present experiment do not indicate that yield and composition were in any way related.

### Summary

A study was made of the influence of fertilizer treatment, climatic condition, stage of maturity, and variety on the percentage of protein, sulfur, cystine, and methionine in dry peas. The addition of sulfur-containing fertilizers increased the total sulfur and the cystine level of the peas, but decreased the protein. The location on the hill (slope) on which the peas were grown significantly influenced the percent of protein in the peas, but not the cystine or methionine in the protein. Neither harvesting date nor variety influenced the cystine or methionine content of the dry pea proteins, but White Canada peas contained more protein than Alaska, or First and Best.

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## BOOK REVIEW

**Organic Chemistry.** By Paul Karrer. Translated by A. J. Mee with translations of additions and revisions to latest German edition by M. F. Darken. xx + 953 pp. Elsevier Publishing Co., Inc., New York, N. Y. 1946. Price \$7.50.

The book under review is the second English edition of the well-known excellent *Lehrbuch der Organischen Chemie* by Paul Karrer which has been widely used by students of organic chemistry since the publication of the first German edition in 1928. The present text is based on the eighth German edition, which appeared in 1942.

The aim of the book is to provide students with a medium-sized textbook surveying the ever-increasing facts and theories of organic chemistry. Special attention has been paid to the description of methods of synthesis and to the determination of structure of organic compounds. Particular emphasis has been placed upon naturally occurring substances and biological topics; and the problems of stereochemistry are discussed in an excellent fashion.

The arrangement of the book is based upon the historical division of organic chemistry into aliphatic, carbocyclic, and heterocyclic compounds. In each section the compounds are regarded as functions of the hydrocarbons and have been arranged, as far as possible, according to their functional groups. There is also a short division dealing with compounds of heavy hydrogen and heavy oxygen and an appendix consisting of 30 tables covering a variety of subjects. These include tables on the production of coal, mineral oil, sugar, and textiles; tables giving the number of structural isomerides of various organic compounds; dissociation constants of organic acids and bases; and other interesting information.

When comparison is made with the first English edition, no extensive modifications are found in the text of the new edition. Most of the changes consist of small additions of new material. In all, about one hundred changes or additions have been made. A large number of these occur in those portions of the book dealing with natural products and biological processes where new discoveries have necessitated additions or modifications. One important contrast between the present and earlier English edition is the placement of greater emphasis upon the electronic concepts of structure resulting in revisions in those portions of the book dealing with structures, reaction theories, etc. The section which deals with the composition and analysis of organic compounds is unchanged. It is felt that this discussion deserves some revision, at least to the extent of employing illustrations of more modern equipment.

Since the material has been skillfully selected and is presented in an interesting fashion, the book should be well received by students of organic chemistry. It is well written and the price is considerably lower than the first English edition.

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# CEREAL CHEMISTRY



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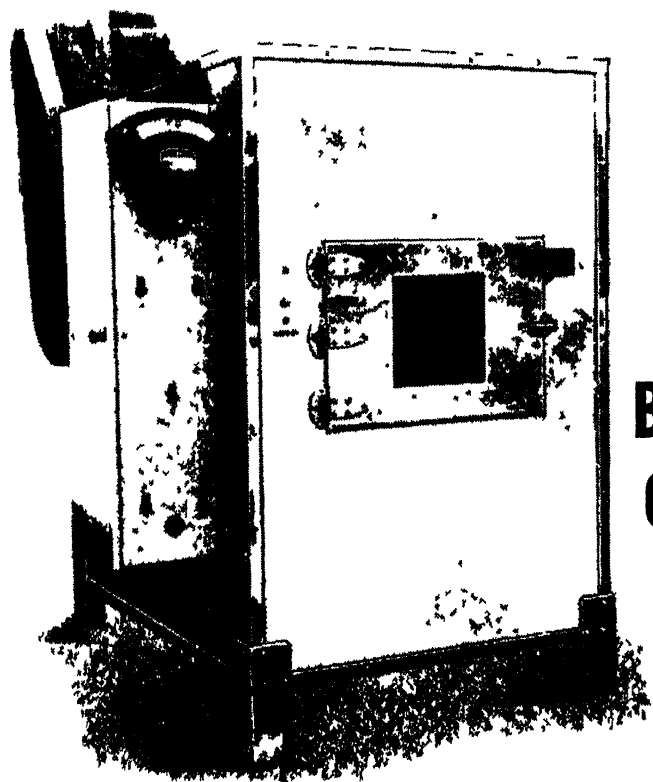
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# CEREAL CHEMISTRY

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No. 4

## STUDIES ON BREAD STALING. I. THE ROLE OF STARCH<sup>1</sup>

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(Presented at the annual meeting, May 1946; received for publication February 24, 1947)

The staling of bread has been of major interest to the starch chemist, partly because starch constitutes the principal constituent of bread and partly because reactions occur in simple starch systems which bear at least a superficial resemblance to staling. During the latter process, the starch substance of bread becomes less soluble, seemingly by spontaneous transformation from a sol state to a crystalline aggregate. It appears that Lindet (1902) originated the term "retrogradation" to describe this change in the starch substance of bread. The same term was later employed by Maquenne and Roux (1905) to designate various insolubilization phenomena of starch itself. These include the gradual increase in viscosity and opacity of starch pastes and solutions, the spontaneous precipitation of starch from solution, and the irreversible gelation of aqueous starch systems. Such effects are explicable on the basis of aggregation of high-polymeric starch molecules through associative forces, to give a particle too large to remain in solution. In support of this theory, Katz (1930, 1934) has shown that staled bread and retrograded starch both possess B-type X-ray patterns, suggestive of side-by-side association of linear starch molecules.

Recent concepts (Meyer, 1942) picture the normal starches as composed of two polymeric carbohydrates, a linear chain of some 400-1000 glucose units (the A-fraction or amylose) and a branched or treelike molecule of perhaps several thousand glucose units (the B-fraction or amylopectin). The first component exhibits the various retrogradation effects of starch to a greatly exaggerated degree; the second component is colloidally stable and shows little evidence of aggregation or insolubilization. Consequently, it has been assumed

<sup>1</sup> This work was conducted as a project under the sponsorship of the Committee on Food Research, Office of the Quartermaster General.

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that the linear component is responsible both for the retrogradation of starch pastes and for the staling of bread. For example, Hixon (1943) speculates that if a waxy variety of wheat were known, bread baked from such a flour might not be susceptible to staling, since the waxy starches contain no linear-chain component.

On the other hand, Alsberg (1927, 1928) has questioned whether staling can be attributed solely to retrogradation, pointing out that staling is a rapid process, while retrogradation is much slower. Also, staled bread is restored to its original freshness merely by heating above 50°C, and the cycle of staling and refreshing can be repeated indefinitely. This behavior is not in accord with the irreversibility usually ascribed to retrogradation of the linear component. MacMasters *et al.* (1946) have observed that pastes of waxy maize starch (containing only the branched B-fraction) become insoluble when frozen and thawed, so that the resulting starch "sponge" does not redissolve at room temperature. Noznick, Merritt, and Geddes (1946) have prepared a synthetic flour from waxy maize starch and non-devitalized gluten; bread baked from this flour showed normal tendency to stale. From these observations it would seem that the linear A-fraction is perhaps not essential to the staling process and that the branched B-fraction by itself may undergo a sort of spontaneous insolubilization.

In view of the major advances in starch chemistry since the studies of Katz, particularly the isolation and characterization of the pure fractions, it seemed advisable to reexamine some of these concepts of bread staling. The purposes of present studies were threefold: (1) to determine which of the starch fractions is responsible for staling, (2) to clarify the mechanisms involved, and (3) to evaluate the influence of other bread constituents (proteins, shortening, salt) on the staling process.

### Procedures

*Nature of the Soluble Starch from Bread.* It is well known that an appreciable amount of the starch in fresh bread is in a soluble state and can be extracted from the crumb with cold water. During staling, the amount of this soluble starch decreases progressively; in fact, this effect has been used as a criterion for estimating the extent of staling (Katz, 1928). At the commencement of present studies, it was felt that a more critical examination of this soluble starch should provide additional information on the changes that occur in the starch substance of bread during the staling process. Consequently, the solubles have been extracted from bread at various stages of staling by leaching with water at 30°C. The amount of this soluble carbohydrate and its

iodine affinity have been determined in order to trace the fate of the individual starch fractions during staling. Incidental to these studies, a modified procedure has been developed for evaluating the swelling power of bread crumb.

Wrapped sliced sandwich bread was obtained locally within a few hours after baking and stored at 21°C and 60% relative humidity. Due to development of mold, tests could not be carried beyond 6 days. After various periods of storage, 25.0-g samples (on as-is basis) were taken for analysis, using the center of the loaf and discarding one-half inch of crust and outer crumb. Simultaneously, a sample was taken for moisture determination, drying for 4 hours in the vacuum oven at 70°C. The 25-g sample was broken up and placed in a tared 250-ml Pyrex centrifuge bottle. Approximately 150 ml of distilled water was added and the mixture stirred vigorously for one minute with a propeller-type stirrer to give a smooth paste.<sup>3</sup> The bottle was placed in a thermostated bath at 30°C for 30 minutes, the contents being gently stirred during this period to keep the bread crumb suspended. The mixture was then centrifuged for 5 minutes at 2000 RPM, the supernate decanted, and the bottle plus sediment weighed. The sediment was resuspended in 100 ml of distilled water and again digested for 30 minutes in the 30°C bath. After centrifuging, the supernate was decanted and the bottle and sediment again weighed. Sediment weights were averaged<sup>4</sup> and the swelling power calculated as the weight of sediment per gram (dry basis) of bread. The sediment was extracted a third time in similar fashion, and the three decantates combined and filtered to remove insolubles and fatty material. The filtrate and wash waters were treated with 3–4 volumes of methanol to flocculate the soluble starch, the mixture heated on the steam bath for an hour, then allowed to settle overnight. The precipitate was collected in a weighed Gooch crucible, washed with alcohol, and dried in the vacuum oven. The percentage of solubles was calculated on the dry-basis bread. To remove traces of fatty material which might interfere with estimation of iodine affinity, the soluble starch was Soxhlet-extracted for 16 hours with methanol. Iodine affinities were determined by a modified potentiometric method.<sup>5</sup> Duplicate extractions were made in each case, and results are summarized in Table I.

<sup>3</sup> An attempt was made to use the Waring Blendor to break up the crumb structure, but this treatment produced extensive rupture of the starch granules. After two minutes of "blending," microscopic examination showed that most of the granules had been disintegrated. Likewise, the action of the "Blendor" caused a progressive increase in the swelling power of the crumb.

<sup>4</sup> In most cases, the agreement between the first and second weighings of the sediment was within experimental error. Precision of measurement of swelling power is of the order of 1% (i.e., average deviation from the mean is 11 parts per 1000).

<sup>5</sup> Method of Bates, French, and Rundle (1943), modified by Wilson, Schoch, and Hudson (1943).



TABLE I

EFFECT OF STALING ON THE SWELLING POWER OF BREAD CRUMB AND THE QUANTITY AND COMPOSITION OF SOLUBLES EXTRACTABLE AT 30°C

Age	Moisture	Swelling power	Solubles	I <sub>2</sub> affinity of solubles	Estimated composition of solubles <sup>1</sup>	
					A-fraction <sup>2</sup>	B-fraction <sup>3</sup>
Hours 4	%	3.61	6.21	1.91	0.60	5.61
			7.94	2.17	0.87	7.07
6	43.6	3.67	7.12	2.04	0.73	6.39
			7.40	2.31	0.86	6.54
28	41.0	3.06	4.74	1.66	0.40	4.34
			4.85	1.63	0.40	4.45
76	39.8	2.72	3.48	1.45	0.25	3.23
			3.65	1.45	0.27	3.38
148	37.3	2.64	3.06	1.53	0.24	2.82
			3.10	1.33	0.21	2.89

<sup>1</sup> Calculated to dry bread basis.<sup>2</sup> Calculated on basis of 19.9% iodine affinity for pure wheat A-fraction, viz., % A-fraction =  $\frac{\% \text{ solubles} \times \text{iodine affinity of solubles}}{19.9}$ .<sup>3</sup> By difference.

It was of interest to ascertain how the refreshing of staled bread by moderate heating might influence the yield and character of the soluble starch. It would be difficult to standardize conditions of refreshing by merely heating the staled bread in an oven, owing to different moisture content of the various samples. Hence refreshing was effected by conducting the extraction of soluble starch at 50°C, using the same procedure as for 30°C. Data on swelling power at 50°C and amount and iodine affinity of the solubles are presented in Table II.

*Fractionation of Wheat Starch.* Since iodine affinity was employed to establish the composition of the soluble extract at various stages of staling, it was necessary to determine the iodine affinity of defatted wheat starch and of pure wheat A-fraction. The wheat starch employed for this purpose was from an experimental batch prepared at the Northern Regional Research Laboratory by SO<sub>2</sub> steeping. Preliminarily, this starch was defatted by four successive digestions with boiling 85% methanol, each such digestion for a period of 3 hours. Its iodine affinity by the modified method of Whistler and Hilbert (1945) was  $5.21 \pm 0.07\%$ . This defatted starch was fractionated by gelatinizing in hot water to give a 2% paste; the latter was autoclaved for 2 hours at pH 6.5, 10% by volume of "Pentasol" added (Sharples

brand mixed amyl alcohols), and the mixture allowed to cool to room temperature with continuous stirring. The crude A-fraction was collected in the Sharples supercentrifuge, dissolved in hot water in the presence of excess n-butyl alcohol, and the hot solution supercentrifuged to remove extraneous impurities. The recrystallized A-fraction separated on cooling; it was repeatedly recrystallized by solution in hot water and precipitation with butyl alcohol, until the iodine affinity reached a maximum value of 19.9%. Experimental yields of A-fraction were not determined with sufficient care. However, the content of linear component can be calculated as 26% from the iodine affinities of the defatted starch and the pure A-fraction (viz., 5.21% divided by 19.9%). Two batches of wheat B-fraction had iodine affinities of 0.56% and 0.44%.

*Nature of the Soluble Starch from 50% Pastes of Defatted Wheat Starch.* Bread represents a highly complex system, in which both physical and chemical interactions may occur between the various components. To avoid such complications, it seemed logical to transpose these studies to simple starch-water pastes. If the swelling power and the amount and iodine affinity of the solubles show the same trends during the aging of simple starch pastes as during the staling of bread, then these effects must be inherent in the starch itself and not due to the influence of other bread constituents.

Consequently, subsequent aging studies were conducted on defatted wheat starch pastes, prepared under conditions to simulate the

TABLE II  
EFFECT OF STALING ON THE SWELLING POWER OF BREAD CRUMB AND THE  
QUANTITY AND COMPOSITION OF SOLUBLES EXTRACTABLE AT 50°C

Age	Moisture	Swelling power	Solubles	I <sub>2</sub> affinity of solubles	Estimated composition of solubles	
					A-fraction	B-fraction
<i>Hours</i>	<i>%</i>		<i>%</i>		<i>%</i>	<i>%</i>
4	42.6	3.75	9.04	3.06	1.39	7.65
			9.64	2.95	1.43	8.21
6	43.6	4.02	7.41	2.73	1.02	6.39
			7.70	2.78	1.08	6.62
28	41.0	3.78	8.04	2.72	1.10	6.94
			8.04	2.80	1.13	6.91
76	39.8	3.68	7.48	2.90	1.09	6.39
			7.89	2.64	1.05	6.84
148	37.3	3.73	7.70	2.56	0.99	6.71
			8.09	2.74	1.11	6.98

baking and cooling of the loaf of bread. The concentration of the paste was arbitrarily fixed at 50% (dry starch basis). Confirming Alsberg's statement (1927, 1928) that all the starch granules of bread are completely gelatinized, observations of moistened bread crumb under the polarizing microscope showed uniform swelling and no birefringent granules. Some difficulty was encountered in effecting similar uniformity in the gelatinization of 50% wheat starch suspensions. When a test tube containing such a suspension was rapidly heated in a boiling water bath, the starch adjacent to the walls of the tube gelatinized immediately, withdrawing water from the interior of the suspension and leaving a core of ungelatinized starch in the center of the tube. However, when the starch suspension was slowly heated, uniform gelatinization of the granules occurred throughout the suspension. To duplicate the temperature rise during the baking of bread, a standard loaf was baked with a thermometer imbedded in the center of the dough. In close agreement with Barackman and Bell (1938), the temperature rose to 100°C over a period of about 20 minutes, then remained at this level for the remaining 20–30 minutes of baking. Similar conditions were therefore used for the pasting of 50% starch suspensions.

An amount of the defatted wheat starch equivalent to 10.0 g on dry starch basis was weighed into a 25 × 100 mm test tube and sufficient water added to give a total weight of 20.0 g (i.e., 10 ml of water less the moisture of the starch). The mixture was thoroughly stirred with a small nickel spatula until homogeneous, the spatula removed, and any adhering starch scraped back into the tube. The latter was stoppered with a vented rubber cap and placed in a water bath, the temperature of which was raised at a uniform rate to 100°C over a period of 30 minutes. The paste was heated in the boiling water bath for an additional 30 minutes, then allowed to cool slowly to room temperature. Under these conditions, microscopic examination showed only a few ungelatinized and birefringent granules, probably representing less than 2% of the total starch.

The resulting gels were hard, elastic, and almost cartilaginous in character. Extraction studies were conducted on these pastes after cooling to room temperature (i.e., 0 days) and after 3 and 6 days' storage at 21°C. To prepare the sample for extraction, the gel (representing 10.0 g of starch) was removed from the tube and pressed through a 40-mesh screen, then rinsed quantitatively into a tared 250-ml Pyrex centrifuge bottle with 150 ml of water. Swelling power, amount of soluble starch, and iodine affinity of the solubles were determined on starch pastes aged for 0, 3, and 6 days, the extractions in each case being conducted at 30°C and at 50°C (Table III). The

technique of extraction was similar to that described above for bread samples, except that the combined decantates were precipitated directly without filtering, and Soxhlet extraction of the soluble starch was omitted.

*Effect of Adjuncts on the Aging of Starch Pastes.* This technique of operating on simple starch pastes permits evaluation of the influence of other bread constituents on the staling process. Similar tests have therefore been made on the aging of 50% defatted wheat starch pastes,

TABLE III  
EFFECT OF VARIOUS BREAD INGREDIENTS ON THE STALING OF 50% WHEAT STARCH PASTES, AS MEASURED BY CRUMB SWELLING POWER AND PERCENTAGE OF SOLUBLES

Adjunct	Age	Extraction at 30°C			Extraction at 50°C		
		Swell	Solubles	I <sub>2</sub> affinity	Swell	Solubles	I <sub>2</sub> affinity
Defatted wheat starch	0	5.05	3.03	3.4	5.37	3.15	4.5
		5.17	2.85	3.5	5.48	3.51	4.5
No adjunct	3	3.63	1.02	3.4	5.16	3.15	3.6
		3.61	1.00	2.9	5.02	3.06	4.0
	6	3.57	1.05	3.3	4.95	2.66	3.7
		3.52	1.04	3.5	5.40	2.74	4.0
Defatted wheat starch+2% salt	0	4.55	2.66	3.7	5.31	3.98	4.1
		4.51	2.54	3.7	5.22	4.68	4.3
	3	3.53	1.11	2.9	5.10	3.00	4.1
		3.54	1.09	3.3	4.87	3.15	4.2
	6	3.53	0.97	3.1	4.72	2.43	4.1
		3.55	0.87	3.0	4.95	2.61	4.1
Defatted wheat starch+2% yeast	0	4.88	2.99	3.6	5.38	4.68	3.8
		4.71	3.10	3.3	5.37	4.47	3.8
Defatted wheat starch+25% egg albumen	0	5.42	3.64	4.2	6.57	4.14	5.5
		6.33	4.12	4.5	6.22	6.36	5.1
	3	5.07	1.55	3.5	6.02	3.33	4.5
		4.90	1.75	3.6	6.01	3.06	5.1
	6	4.74	1.19	3.8	5.88	2.89	4.5
		4.84	1.26	4.1	5.90	2.75	4.5
Defatted wheat starch+10% casein <sup>1</sup>	0	4.58	2.57	4.0	4.99	3.38	5.8
		4.48	2.44	2.0	5.12	3.75	5.0
	3	3.44	0.88	3.9	4.77	2.91	5.2
		3.48	1.00	4.0	4.85	3.20	4.8
	6	3.48	1.45	1.2	4.55	3.69	3.5
		3.62	2.26	1.8	4.52	3.47	4.0

TABLE III—*Continued*

Adjunct	Age	Extraction at 30°C			Extraction at 50°C		
		Swell	Solubles	I <sub>2</sub> affinity	Swell	Solubles	I <sub>2</sub> affinity
Defatted wheat starch +4% hydrogenated shortening	Days 0	4.32	2.59	3.3	4.81	3.67	4.9
		4.33	2.57	4.1	4.96	3.42	4.0
	3	3.67	0.91	3.7	4.54	2.53	4.3
		3.55	0.97	3.2	4.51	2.43	4.9
	6	3.54	0.79	2.9	4.58	2.37	5.1
		3.48	0.76	2.6	4.55	2.61	3.8
Defatted wheat starch +4% corn oil	0	4.91	2.94	3.2	5.51	3.60	4.7
		4.79	3.07	2.9	5.60	3.25	4.4
	3	3.70	1.23	2.1	4.93	2.79	3.0
		3.62	1.14	1.9	4.87	2.73	3.0
	6	3.54	0.89	0.7	4.64	2.49	2.9
		3.57	0.90	0.9	4.66	2.53	2.8
Defatted wheat starch +2% oleic acid	0	3.83	1.29	0.5	4.09	2.25	<0.2
		3.81	1.54		4.05	1.68	
	3	—	—	—	3.94	1.83	2
		—	—	—	3.91	1.79	2
	6	3.22	1.17	2	3.88	1.99	2
		3.17	1.20	2	3.88	1.96	2
Defatted wheat starch +0.2% malt extract	0	3.49	11.09	1.6	4.12	11.44	1.8
		3.32	10.65	1.6	3.92	11.94	1.9
	3	3.14	6.95	1.4	3.89	12.58	1.8
		3.16	6.66	1.5	4.05	12.21	1.8
	6	3.22	4.11	1.7	4.16	9.84	1.8
		3.24	3.95	1.6	4.18	9.66	1.6

<sup>1</sup> Nitrogen was determined on each extract by a micro-Kjeldahl procedure. Values for % solubles represent total solubles minus the computed protein. Iodine affinities are corrected to starch basis.

<sup>2</sup> These samples were not analyzed for iodine affinity, since they gave a violet or red color with iodine, indicating an iodine affinity less than 0.6%.

but with the incorporation of various adjuncts to simulate the non-starchy constituents of bread. Details of processing are as follows:

**SALT (2%):** Pastes of defatted wheat starch were prepared as described above, except that 2% sodium chloride (on dry starch basis) was added to the suspension before pasting.

**YEAST (2%):** Dry yeast was incorporated in the mixture before pasting, to the extent of 0.2 g per 10 g of starch. Since the pastes did not show any substantial difference from defatted wheat starch, tests were not conducted beyond zero days.

**EGG ALBUMEN (25%):** Fifty g of "soluble egg albumen" was stirred into 200 ml of water, the mixture allowed to stand overnight in the refrigerator, then centrifuged to remove insolubles, and the pH adjusted to 6.5 with potassium hydroxide solution. Ten g (dry basis) of defatted wheat starch was mixed with 12.5 g of this albumen solution, and the mixture gelatinized, aged, and extracted as previously described. The egg albumen was completely insolubilized during the pasting operation and the aqueous extracts at both 30° and 50°C were substantially nitrogen-free.

**CASEIN (10%):** Ten g of acid-precipitated casein was dissolved in 100 ml of water and the pH adjusted to 6.3 with potassium hydroxide solution. Eleven g of this solution was mixed with 10.0 g (dry basis) of defatted wheat starch and the mixture pasted. Since the casein was not insolubilized by heat, it was extracted in the solubles and precipitated by alcohol. Kjeldahl analyses were therefore run on the extracted solubles and the starch calculated by difference. The accuracy of such a procedure is questionable, but there was no feasible method for effecting a quantitative separation between soluble starch and casein. Iodine affinities were determined on the total solubles and corrected to starch basis. Separate tests showed that the iodine affinity of wheat A-fraction is not appreciably affected by the presence of dissolved protein (i.e., casein or gelatin) in quantities as high as five times the amount of A-fraction being analyzed.

**SHORTENING (4%):** Six g of hydrogenated vegetable shortening was dissolved in 150 ml of hexane, thoroughly mixed with 150 g of defatted wheat starch, then the hexane removed on the steam plate with frequent stirring. Moisture content of the mixture was determined and pastes made up to contain 10.0 g starch, 0.4 g shortening, and 10 g water. Prior to evaluation of iodine affinity, the soluble starch was extracted overnight with ethyl alcohol.

**CORN OIL (4%):** Twelve g of refined corn oil and 300 g of defatted wheat starch were blended for one hour in a Hobart mixer. Subsequent procedure duplicated that employed with hydrogenated vegetable shortening.

**OLEIC ACID (2%):** This amount was sufficient to form an insoluble complex with all of the A-fraction of the wheat starch (Schoch and Williams, 1944). Three g of oleic acid was blended into 150 g of defatted wheat starch, and the pastes made up to contain 10.0 g starch, 0.2 g oleic acid, and 10 g water. The soluble starch was Soxhlet-extracted for 36 hours with ethyl alcohol. Due to low iodine affinity, it was necessary to combine the duplicate solubles from zero days aging, to provide sufficient material for iodine titration. The solubles from 3- and 6-day old pastes were tested qualitatively in each case

and found to give a red to red-violet color with iodine, indicative of negligible iodine affinity.

**MALT EXTRACT (0.2%):** 0.4 ml of diastatic malt extract (250° Lintner) was dissolved in 200 ml of water. 10.0 g of defatted wheat starch was then pasted with 10 ml of this solution. Judging from Lintner value, this enzyme level probably exceeds that of a diastated flour.

### Discussion of Results

In order to interpret results on the extraction of bread, it is necessary to recall the classical fractionation of starch by selective leaching methods. When an aqueous starch suspension is heated to a temperature just sufficient to gelatinize the granules (as indicated by loss of birefringence) without excessive swelling, a portion of the A-fraction dissolves and diffuses into the aqueous substrate. If the swollen granules are then removed by sedimentation or centrifugation and the soluble carbohydrate precipitated from the supernate with alcohol, this material (termed the "amylose" by K. H. Meyer) will have an iodine affinity as high as 14%, indicating a preponderance of the linear-chain A-fraction. Correspondingly, the insoluble residue of swollen granules (Meyer's "amylopectin") will have an iodine affinity lower than that of the whole starch (Schoch, 1945).

It might be expected that a similar situation would occur during extraction of freshly baked bread, whereby the A-fraction would be leached from the swollen but intact granules. However, the iodine affinity of the solubles from fresh bread (Table I) is far below that of defatted wheat starch (i.e., 5.21%). The A-fraction must therefore be rendered nonavailable during baking and cooling of the loaf, probably by one or more of the following reactions:

1. The A-fraction may retrograde to insoluble state during baking of the loaf, thereafter resisting aqueous extraction.
2. The A-fraction may be immobilized as an insoluble complex with other bread constituents, such as fat or protein.
3. The A-fraction may be hydrolyzed by flour enzymes during the early stages of baking (e.g., after the temperature is sufficiently high to gelatinize the granules but insufficient to inactivate the enzyme).

This behavior is not peculiar to bread, since the solubles from freshly prepared 50% pastes of defatted wheat starch likewise have an iodine affinity lower than that of the parent starch (Table III). In the absence of fat, protein, and enzymes, this effect can only be attributed to retrogradation of the A-fraction during pasting and cooling of the

starch paste. Similarly, most of the A-fraction of fresh bread must retrograde during baking and cooling of the loaf. It seems unlikely that the very minor amount of A-fraction still in available soluble form could be responsible for subsequent staling. The soluble starch from fresh bread is greater in amount and lower in iodine affinity than the solubles from 50% starch pastes. At a later point, these differences will be attributed to the influence of flour enzymes and shortening.

The percentage of solubles extractable at 30°C decreases progressively with staling of the bread (Table I). There is a slight decrease in iodine affinity, indicating retrogradation of the small amount of A-fraction still in solution. However, this effect is considered negligible. The more important action is a progressive decrease in the amount of B-fraction leached from the bread, indicating a spontaneous insolubilization of this material during staling. Here, again, the same trend is duplicated with 50% pastes of defatted wheat starch (Table III); hence the effect is not due to the influence of other bread constituents. This behavior suggests (1) that the staling of bread is due to a progressive spontaneous aggregation of the branched-chain component and (2) that the linear fraction has no influence on staling, since it is already in an inert retrograded form.

This theory receives added confirmation from extraction studies at 50°C. While it was later realized that a somewhat higher temperature (e.g., 55°C) might have given more ideal results, the "refreshening" of both staled bread (Table II) and staled starch pastes (Table III) is obvious at 50°C. The swelling power and percentage of solubles are restored to high and fairly constant levels. The iodine affinity of the solubles, while higher than values obtained by extraction at 30°C, is still substantially lower than that of defatted wheat starch. Thus, solubilization of retrograded A-fraction cannot play any important part in the refreshing of staled bread.

If spontaneous insolubilization of the B-fraction is responsible for the decrease in swelling power and solubles on aging of starch pastes, then these effects should be observed with waxy maize starch. However, freshly prepared 50% pastes of defatted waxy maize starch were glutinous and slimy and could not be processed by the analytical procedures employed for bread and for 50% wheat starch pastes. On aging for 3-6 days, these pastes were transformed into hard tough gels, of much the same character as 6-day-old wheat starch pastes. When these staled gels were heated to 100°C, they reverted to slimy pastes. Hence the phenomena of staling and refreshing are duplicated at least qualitatively with waxy maize starch, containing no A-fraction.

While the solubles from bread and from 50% starch pastes show the same qualitative trends during staling, there are two outstanding



quantitative differences. The bread solubles are much greater in amount and substantially lower in iodine affinity than the solubles from a comparable starch paste. It seems probable that these differences are due to the influence of other bread constituents on the starch substance. Such hydrophobic polar materials as cyclohexanol or the higher fatty acids profoundly affect the pasting behavior of starch. These agents restrict granule swelling and likewise prevent leaching of the A-fraction. For example, if a dilute starch suspension is saturated with cyclohexanol and autoclaved at 123°C, the granules are only moderately swollen and no substantial amount of carbohydrate dissolves in the aqueous substrate. Similar effects are observed when starch is leached with water in the presence of excess fatty acid or cyclohexanol. The A-fraction is bound as an insoluble complex with the polar agent; the soluble extract is therefore small in amount and has an iodine affinity lower than that of the parent starch. Even the small amount (0.65%) of fatty acid normally present in corn starch raises the gelatinization temperature, restricts granule swelling, and impedes solution of the A-fraction. Only 5% of soluble amylose can be leached from nondefatted corn starch by water at 70°C (Kerr and Severson, 1943), as compared with 14% from defatted starch.

The use of simple wheat starch pastes permits evaluation of the separate influence of other bread constituents (Table III). The addition of 2% oleic acid markedly reduces the swelling power, amount of solubles, and iodine affinity of the solubles, even with freshly prepared pastes. It might be anticipated that a mono- or diglyceride would have a similar action. Shortening itself has little effect unless fatty acid develops by rancidification. The amount and iodine affinity of the soluble starch from bread must therefore be conditioned by natural

TABLE IV  
COMPARATIVE EXTRACTION DATA FOR BREAD AND FOR MALTED STARCH  
PASTES (CALCULATED TO DRY STARCH BASIS)

	Age	Extraction at 30°C		Extraction at 50°C	
		Solubles	I <sub>2</sub> affinity	Solubles	I <sub>2</sub> affinity
Bread <sup>1</sup>	<i>Days</i>	%		%	
	0	9.6	2.1	11.9	2.9
	3	4.8	1.5	10.3	2.8
	6	4.1	1.4	10.5	2.7
Malted starch paste	0	10.9	1.6	11.7	1.9
	3	6.8	1.5	12.4	1.8
	6	4.0	1.7	9.8	1.7

<sup>1</sup> Data for bread averaged from Tables I and II and calculated to starch basis.

lipids of the wheat starch and by fatty acids developed in the shortening.

Natural or added enzymes in the flour may likewise have a modifying influence on the yield and character of the soluble starch. When malt extract is added to the starch prior to pasting, the amount and iodine affinity of the solubles closely parallel the values for bread. This similarity is particularly striking if the data for bread are calculated to a dry starch basis, assuming that the bread contains 75% starch (Table IV). Presumably the solubles are increased by alpha-amylase action during the early stages of baking.

Salt or yeast has little influence on the staling of 50% starch pastes. Some evidence of an insoluble starch-protein complex was anticipated, but neither casein nor egg albumen (exemplifying heat-stable and heat-coagulable proteins) has any very substantial effect on the staling of starch pastes. If anything, egg albumen appears to retard staling to a slight degree.

### Aggregation of the B-Fraction of Starch

Oriented aggregation of the linear A-fraction is a well-established phenomenon, as shown by X-ray studies on the retrograded A-fraction and on stretched films of its acetate. Aggregation of the branched B-fraction is less clearly defined, but certain of the more obscure reactions of starch can perhaps be ascribed to such a cause. Meyer and Bernfeld (1940) have attributed the integrity of the granule and its pasting behavior to the interlocking structure of the amylopectin (or B-fraction), whereby the branches of neighboring molecules are organized into intermolecular areas of crystalline association ("fringe micelles"). The gradual relaxation of associative forces in these crystalline areas at elevated temperature is offered as explanation for the swelling of the granule. Sair and Fetzer (1944) have observed that potato starch undergoes a remarkable transformation when heated to 100°C in the presence of moderate amounts of moisture (i.e., 20-40% on dry starch basis). While the potato starch granule retains its birefringence under these conditions, its X-ray pattern passes from the B-pattern of a tuber starch to the A-pattern usually associated with the cereal starches. The gelatinization temperature is raised by this so-called "heat-moisture treatment," and the character of the paste more closely approaches that of the common cereal starches. It might be speculated that the branched B-fraction is present in the potato starch granule in less aggregated form than in the cereal starches, and that heating in the presence of limited amounts of water permits rearrangement into a more highly oriented association. A major difference between the tuber and cereal starches may arise from the fact that the

latter have been naturally "heat-moisture treated" during ripening of the grain. Observations in this laboratory show that the gelatinization temperature of immature (milk-stage) corn starch is some 8°–10°C lower than that of the mature corn starch. This might be interpreted to mean that the degree of association within the granule increases with maturation of the grain. MacMasters *et al.* (1946) have observed that when pastes of waxy maize starch are frozen and thawed, the starch loses its pastelike character and becomes insoluble at room temperature. From microscopic studies, they have further observed that this transformation appears to be accomplished through the formation of a "coacervate." Kruyt (1930) has applied the latter term to the separation of a colloid from solution as an emulsoid of liquid droplets that may subsequently coalesce to give a discrete layer. The decreased solubility of the starch coacervate suggests that some sort of intermolecular association has been established.

A further example of aggregation has been observed in this laboratory. When small amounts (10–20% by volume) of the lower alcohols are added with stirring to a 2–3% solution of corn B-fraction at room temperature, the solubility of the B-fraction is not markedly affected. Even after standing for several days at room temperature, the clarity of the mixture is not visibly different from that of the original solution of B-fraction. But if the mixture is refrigerated overnight at 3°–5°C, the B-fraction is quantitatively precipitated from solution. This precipitate is insoluble at room temperature and may be filtered on a Buchner and washed with water. The physical form of the precipitated B-fraction under the microscope depends on the amount and type of alcohol employed. Thus, 10% addition of methanol gives a microscopic floc, 20% methanol gives spherules approximating the size of the corn starch granule, and 30% gives small spherules the size of rice starch granules. Ethyl, propyl, isopropyl, sec.-butyl, isobutyl, tert.-butyl, and tert.-amyl alcohols all give microscopic flocs at concentrations from 10% to 20% by volume.<sup>6</sup> In no case does precipitation of the B-fraction occur at 5% concentration of any of these alcohols. Above 30% alcohol concentration, the precipitates begin to assume the doughy character of B-fraction when flocculated by gross addition of alcohol; this type of product can be dissolved in water at room temperature.

<sup>6</sup> This behavior has been utilized to effect a separation of the starch fractions by precipitation at different temperature levels. If a hot autoclaved starch solution is treated with 15–35% by volume of

effected if sufficient alcohol is employed so that the concentration of dissolved alcohol in the refrigerated solution of B-fraction is at least 10% by volume. Thus n-butyl alcohol cannot be employed, since it is not sufficiently soluble in water at 0°C to cause flocculation of the B-fraction. Obviously the alcohol concentration must not exceed 40% or the entire starch substance will be immediately precipitated. Preferred systems include 25% isopropyl alcohol and 18–20% sec.-butyl alcohol.

When these precipitates are slowly heated in water medium, their insolubility persists until the temperature reaches approximately 50°C, when the product tends to dissolve. This solubilization can be followed most readily under the microscope with the large spherules obtained by refrigeration with 20% methanol. If these are suspended in water and heated at a gradually rising temperature (most conveniently on the Kofler micro hot stage), there is no change in the size and shape of the spherules until the temperature reaches 52°C. At this point the spherules commence to swell, in a manner very similar to the swelling of a starch granule. Swelling is progressive up to 70°C, when the greatly swollen spherules begin to rupture and their outlines become vague. It should be remarked that no optical anisotropy has been observed with any of these B-fraction precipitates, either spherules or micro flocs. Through the courtesy of Dr. R. E. Rundle of Iowa State College, X-ray diffraction patterns have been obtained from the undried precipitates formed by refrigeration with 10%, 20%, and 30% additions of methanol. He reports that these products yield, respectively, a fairly prominent B-pattern, a faint B-pattern, and an amorphous diagram. Hence it appears that some sort of orderly crystallization of the B-fraction occurs, rendering that substance insoluble in water except at elevated temperatures. As an extension of these observations, the swelling behavior of starch must be attributed to organization within the granule and not to the presence of any exterior "membrane."

There would seem to be a close parallel between this type of crystallization and the staling of bread. Both reactions are most pronounced at temperatures around freezing, both give rise to a B-type X-ray pattern, both tend to reverse at temperatures above 50°C. As a tentative hypothesis, it is suggested that the following reactions typify aggregation of the branched B-fraction:

1. Staling of bread
2. Staling of 50% starch pastes
3. "Coacervation" by freezing
4. Precipitation by refrigeration with alcohol
5. Granular structure and pasting behavior of starches, especially those of waxy type

The primary conditions which seem to be requisite for this change are (1) a limited amount of water to permit orientation of the branches of the B-fraction and (2) relatively high concentrations of B-fraction. With respect to the first condition, it is known that crackers stale only when their moisture content becomes too high. Similarly, the starch

industry has repeatedly observed that pregelatinized starches dried on hot rolls tend to lose their cold water dispersibility (a "staling" reaction) when stored at high moisture levels. With respect to the second condition for aggregation, the B-fraction has gained its reputation for colloidal stability mainly on its behavior in dilute solutions. In each of the five instances cited above, the B-fraction either is in concentrated solution or else passes through such a phase. Thus, it is progressively deprived of water by freezing, it is laid down in the maturing grain at high concentrations, it appears to separate from water-alcohol medium as an emulsion of highly concentrated liquid droplets which then undergo crystallization. A starch coacervate might be regarded as a somewhat disorderly "brush-pile" of molecules, rendered insoluble by reason of molecular entanglement and associative cross-linkage. Crystallization of such a coacervate may occur if the colloidal substance has sufficient linearity and if conditions permit a certain freedom of orientation. An emulsion of lauryl alcohol in water provides an excellent analogy of this secondary crystallization. Rapid cooling gives a random crystallization of lauryl alcohol leaflets throughout the emulsion droplet. With slow cooling, the droplet is transformed into a perfect spherocrystal, exhibiting a much more brilliant polarization cross than any starch granule.

Methylation analysis, as well as recent end-group assay by periodic acid oxidation, indicates the branch lengths of most of the common B-fractions to be of the order of 20-30 glucose units. This would therefore constitute the effective branch length undergoing this type of aggregation. On this basis, retrogradation of the A-fraction differs only by virtue of its much longer chain length (400-1000 glucose units) and the consequent higher order of association. In contrast, animal glycogen is reputed to be less susceptible to insolubilization, in agreement with its estimated branch length (from end-group assay) of only 11 glucose units (Meyer and Fuld, 1941). The so-called "glycogen" from sweet corn (Morris and Morris, 1939) does not possess sufficient associative tendency to permit its organization into granular form. When a solution of this "corn glycogen" is frozen and then allowed to thaw, it shows no tendency to become insoluble. Similarly, the present writers have observed that the beta-amylase limit dextrin from waxy maize starch<sup>7</sup> is not insolubilized either by freezing or by refrigeration with 10-20% methanol. The same is true of the low-substituted ethyl and hydroxyethyl ethers of starch and (to a lesser degree) of commercial hypochlorite-oxidized corn starches. Presumably the side-branches of these products have been removed or

<sup>7</sup> The limit dextrin was furnished through the courtesy of Miss Edna Montgomery, Northern Regional Research Laboratory, Peoria, Illinois.

else so warped by chemical modification that they are no longer capable of side-by-side association.

It is conceivable that such products (especially the beta-amylase limit dextrin) may have practical utility as antistaling agents for bread. This would perhaps depend on the site of staling in the crumb. It would be difficult to prevent staling within the individual swollen granules by the addition of any such adjunct, since the latter could not penetrate into the gel lattice of the swollen granule. However, the use of such adjuncts in relatively small proportions might prevent cementing between the granules, which may be of even greater importance in the consumer's evaluation of staling. It should be recalled that the latter does not correspond with chemical criteria of staleness. The consumer generally considers 2-day old bread as fairly fresh, though swelling power and percentage solubles would rate such bread as highly staled.

The authors wish to express their thanks to Dr. W. F. Geddes for his helpful comment and advice during the course of this work.

### Summary

The water-soluble starch leached from the crumb of fresh bread at 30°C is predominantly the branched B-fraction, as evaluated by potentiometric iodine titration. The linear A-fraction of the starch must be insolubilized by retrogradation during baking and hence cannot contribute to the staling process. The progressive decline in solubles during the aging of bread indicates that the cause of staling may be a spontaneous aggregation of the branched B-fraction. As additional evidence for this theory, the percentage of soluble B-fraction from staled bread is restored to a constant high level when leached at 50°C; this is in accord with the refreshing of bread by moderate heating. No substantial solubilization of the A-fraction occurs at 50°C. ✓

Parallel behavior is observed during the aging of simple 50% aqueous pastes of defatted wheat starch, prepared under conditions to simulate the baking of bread. This further substantiates the theory that staling is inherent in the B-fraction, and not due to interaction between the starch and other bread ingredients. However, the behavior of the starch substance in bread and the nature of the staling action may be considerably influenced by the presence of other bread constituents. This is shown by a study of the solubles leached from simple starch pastes prepared with the incorporation of various individual bread constituents. Thus malt extract greatly increases the amount of soluble starch and decreases its iodine affinity; free fatty

acid depresses both the amount and iodine affinity of the solubles. Normal amounts of salt, yeast, neutral shortening, casein, or egg albumen have little influence on the staling of 50% starch pastes. It is assumed that these latter constituents will play a minor role in bread staling.

Pure B-fraction undergoes aggregation effects which may typify its behavior in bread staling. If 10–30% alcohol is added to a solution of B-fraction and the mixture refrigerated, the B-fraction precipitates as a floc or as minute spherules, insoluble in water at room temperature and possessing a B-type X-ray pattern. The spherules commence to gelatinize in water at 52°C, undergoing much the same swelling as natural starch granules. It is suggested that the various aggregation phenomena of the B-fraction (including staling) represent an oriented association between the branched chains of the B-fraction molecules. No such aggregation is observed when these branches are removed or modified, as with the beta-amylase limit dextrin, water-soluble starch ethers, and hypochlorite-oxidized starches.

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# STARCH GELATINIZATION STUDIES. I. SIMPLIFIED EQUIPMENT FOR THE STUDY OF STARCH GELATINIZATION BY MEANS OF LIGHT TRANSMISSION<sup>1</sup>

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There are a number of methods available for the study of starch gelatinization. These have been reviewed by Radley (1943). A method that appears to be simple and easily controlled, and that also gives excellent replication of results, is the one based on the measurement of light transmission through the gelatinizing starch dispersion (Cook and Axtmayer, 1937). The equipment used by Cook and Axtmayer was improved by Küntzel and Doehner (1939) and by Morgan (1940). However, these were special setups of a light source and a photoelectric cell. Since apparatus for measuring light transmission (the spectrophotometer) is common laboratory equipment, it seemed that the study of gelatinization by means of light transmission could be considerably simplified by adaptation to this equipment. The present paper describes one such adaptation which has been found convenient for the study of starch gelatinization in this laboratory.

## Equipment and Method of Operation

The spectrophotometer used for this work was a Coleman Model 11.<sup>2</sup> The cuvette carrier, originally designed to hold two cuvettes, was adapted to carry a U tube by removing the partition between the two cuvette openings.

The gelatinizing equipment consists of a U tube (with dimensions as shown in Figure 1) with a bridge (A) between the two arms. The arms are wrapped (as shown in Figure 1) with 160 cm of B and S No. 26 Chromel resistance wire and then insulated by wrapping with asbestos cord. A screw-type glass stirrer is used in one arm of the U tube and a thermometer in the other.

When in operation, the stirrer is turned by a variable speed motor at a rate which circulates the liquid rapidly enough to keep un-

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<sup>2</sup> It is recommended that the spectrophotometer be equipped with a constant voltage regulator for direct line operation. Both the spectrophotometer and voltage regulator should be grounded. If a battery is used, it must be in first-class condition since the galvanometer can be adjusted only at the beginning of a 35-minute run; the light intensity must stay constant during this period.

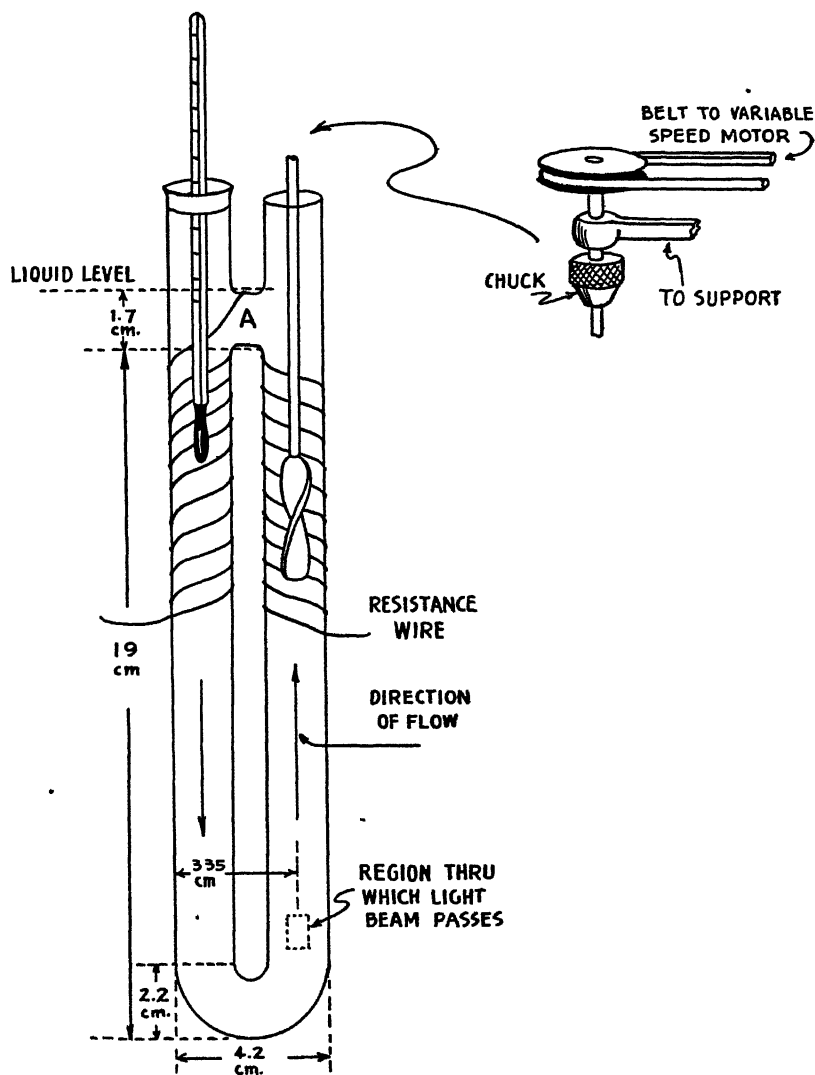


Fig. 1. U tube equipped for the study of gelatinization by means of light transmission.

gelatinized starch in suspension. However, the stirring should not be vigorous enough to cause air bubbles to be entrapped and circulated. By circulating the liquid upward in the arm through which the spectrophotometer light beam passes (as indicated by the arrows in Figure 1), the entrapment of air is avoided.

Rate of heating is controlled by a variable transformer. Morgan (1940) used a rate of  $2.5^{\circ}$  per minute. Cook and Axtmayer suggested

that a slower rate (1.5 to 2.0 minutes for each degree C rise in temperature) was preferable.

For light transmission studies, the ungelatinized starch suspension must be dilute enough to give some transmission of the light beam so that the first changes in gelatinization are observable. Cook and Axtmayer added suspended starch to water in the reaction vessel until the microammeter gave a reading of 3 to 5 microamperes. Morgan used 0.5% dispersions which, with his equipment, gave readings between 5 and 15 microamperes.

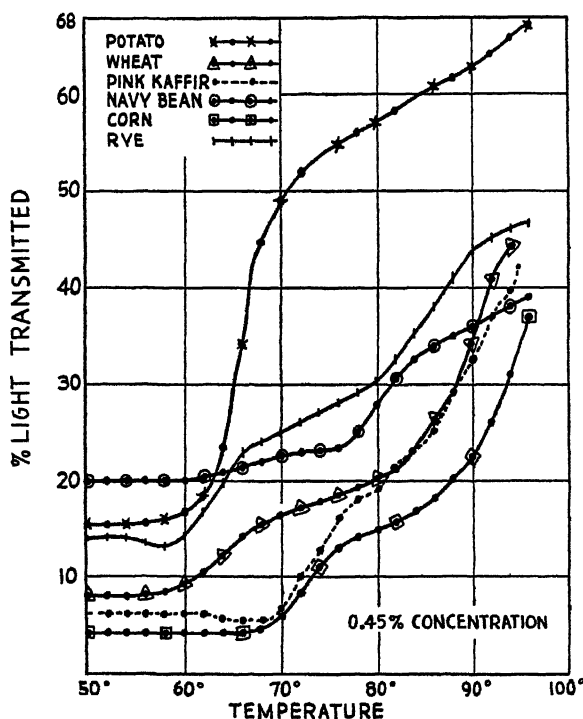


Fig 2 Light transmission curves of 0.45% concentrations of some common starches.

In operation, the U tube is filled with enough water just to cover the bridge (A of Figure 1)<sup>3</sup> and is placed in position in the spectrophotometer. The stirrer is started and the galvanometer is adjusted to read 100% light transmission. Dry starch is introduced through a funnel onto the surface of the circulating liquid. As soon as the galvanometer gives a constant reading for percent light transmission, indicating complete dispersion of the starch, heating is started and maintained at the desired rate. Light transmission readings may be

<sup>3</sup> The tubes described in this paper require 66 ml.

made at any desired interval; at critical periods the readings should be frequent.

### Discussion of Light Transmission Curves

Figure 2 shows gelatinization curves obtained from 0.45% dispersions of some common starches heated at a rate of 2°C per minute. These curves are similar to those shown by Cook and Axtmayer (1937) and by Morgan (1940). They show the wide variation in gelatinization characteristics which is found among different starches.

*The Effect of Varying Starch Concentration.* Figure 3 shows gelatinization curves for wheat starch obtained by heating three concentra-

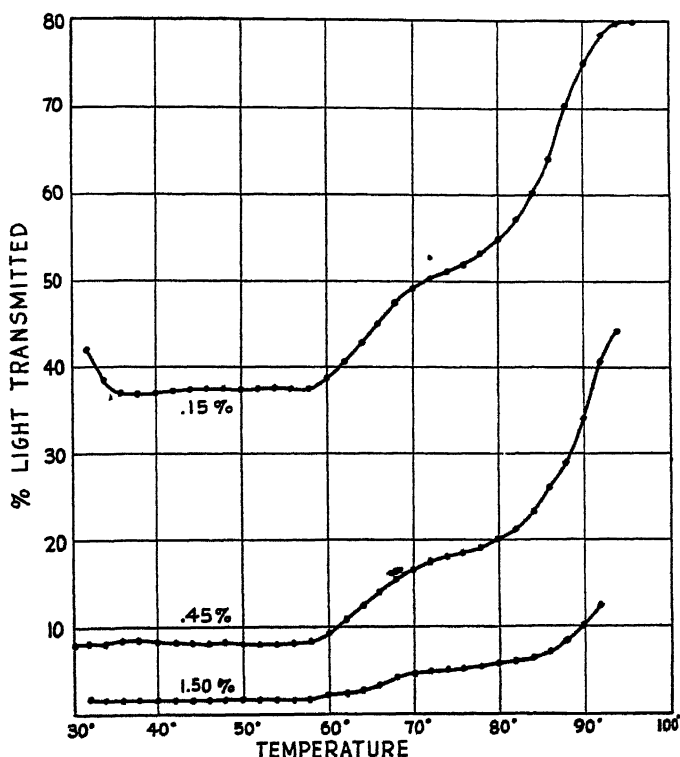


Fig. 3. Effect of wheat starch concentration on light transmission curves.

tions of wheat starch at the rate of 2°C per minute. These data indicate that the gelatinizing characteristics of starch are more clearly shown by the use of the lower concentrations. Since spectrophotometer determinations are most accurate on solutions giving 10% to 60% light transmission, it is well to use a concentration of starch which gives curves falling largely within these limits.

Figure 4 shows data obtained on the same starches as shown in Figure 2, but using 0.20% dispersions. The curves of Figures 2 and 4 are similar in character; however, the curves of Figure 4 give more detail and emphasize the differences among the starches before gelatinization and in the early stages of gelatinization.

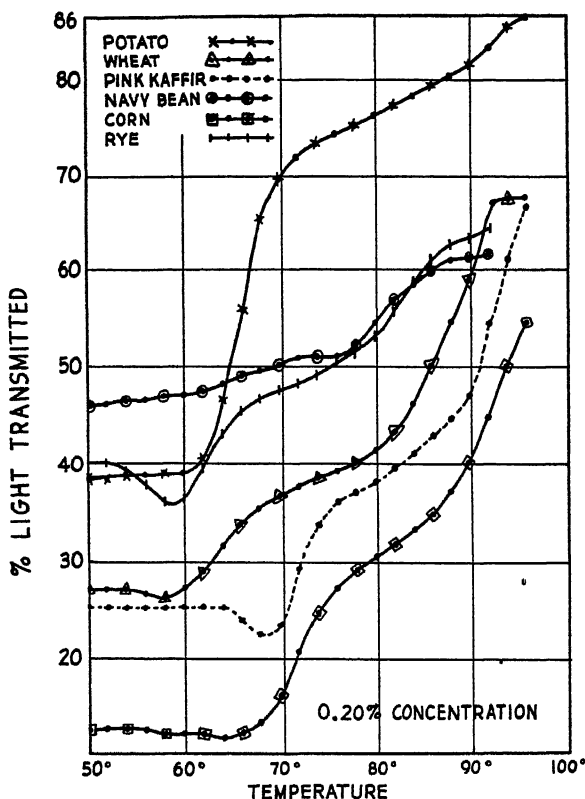


Fig. 4. Light transmission curves of 0.20% concentrations of starches. Compare with Figure 2.

*The Effect of Varying the Rate of Heating.* Cook and Axtmayer (1937) state that the rate of heating affects the "temperature of transition" (the first indication of gelatinization) but does not change the slope of the curve. They found the temperature of transition of cassava starch to be shifted 3°C higher by increasing the rate of heating from 0.16°C to 1.25°C per minute. However, in this laboratory, using the equipment as described, wheat and corn starch gelatinization curves have given excellent replication even when the rate of heating has been varied from 2.5°C to 0.15°C per minute. No significant change could be noted in the "temperature of transition."

Differences in light transmission among native unaltered starches at temperatures below those at which gelatinization begins are due to differences in granule (or aggregate) size (Morgan, 1940); the smaller granules give less light transmission. A decrease in light transmission at the beginning of a curve, as in the upper curve of Figure 3, is due to incomplete dispersion; whereas a dip in the curve at the beginning of gelatinization, as shown by the kaffir and rye starches in Figure 4, may be attributed to disintegration of aggregates of granules (Morgan, 1940). Unpublished work, using other methods of studying gelatinization, indicates that the beginning of the dip coincides with the beginning of gelatinization

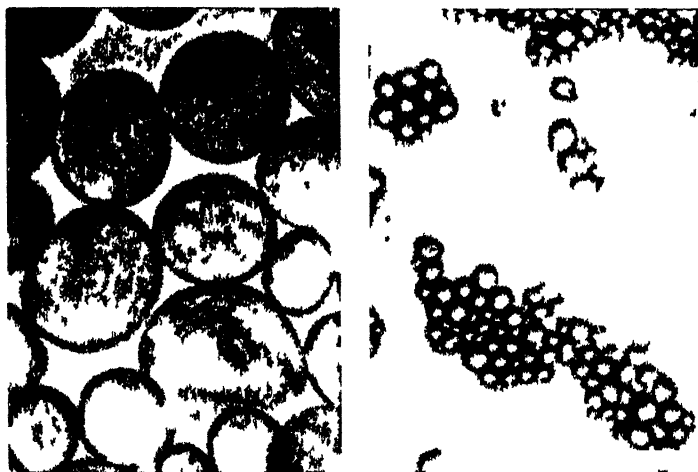


Fig 5 Preparations of large and small granule wheat starch ( $\times 600$ )

Alsberg and Rask (1924) showed by viscometric methods that gelatinization of wheat and maize starches was a gradual change taking place over a range of  $25^{\circ}$  to  $30^{\circ}$ . They pointed out that if the temperature of gelatinization were defined as that at which anisotropy disappears, it must be regarded only as an early stage of the entire gelatinization process. Light transmission curves not only lend emphasis to these observations but the change in rate of gelatinization at certain temperatures suggests that gelatinization takes place in steps (see the curves of wheat, corn, rye, and kaffir, Figure 4). Katz (1928) stated that "for a long time authors have described two grades or stages of gelatinization." Steps in the gelatinization process as shown in Figure 4 correspond to Katz' grades. The first step corresponds to the generally accepted gelatinization temperature (variously defined as that temperature at which the granules swell, begin to lose

starch to the solvent, lose their anisotropy, lose their resistance to staining by certain dyes, etc.), whereas with some starches the second step takes place at considerably higher temperatures.

Morgan (1940) suggested that the changes in gelatinization rate occurring at certain temperatures, as shown by his gelatinization curve of wheat starch, represented the gelatinizing temperatures respectively

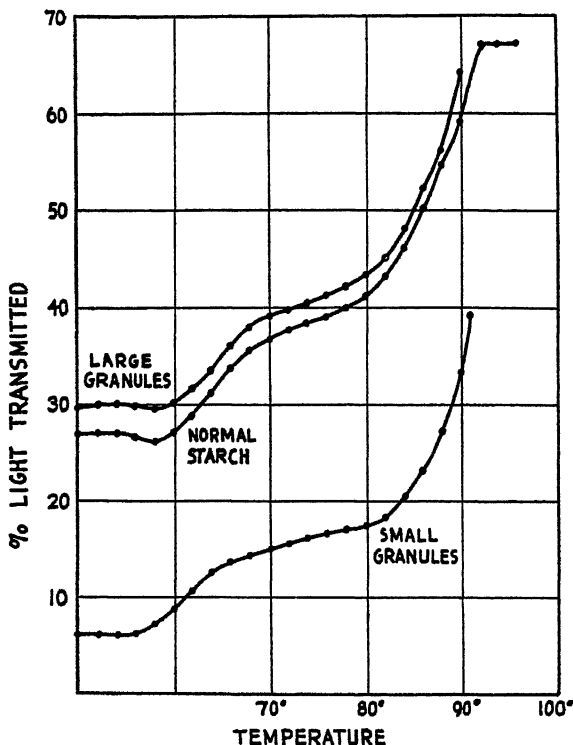


Fig. 6. Effect of size of granule on the light transmission curves of wheat starch.

of the large and small granules. Since the large and small granules may be separated by repeated centrifuging, this supposition may be readily checked.<sup>4</sup>

The photomicrographs in Figure 5 show the character of the large and small granule wheat starch preparations separated by centrifuging. A comparison of the light transmission curve of the original mixture

<sup>4</sup> It is exceedingly difficult to free the small starch granules from the nongranular gelatinous material which consists largely of the dextrins resulting from beta-amylase action on starch damaged in the milling process. This has led some investigators to attribute properties to the small granule starch (MacMasters and Hübner, 1944) which our data indicate are due to nongranular contaminants. In this laboratory the best small granule starch preparations have been obtained by first centrifuging out and discarding the "amylodextrin" fraction. This results in the loss of much small granule starch; however, it is difficult to purify satisfactorily this starch, not only from the gelatinous contaminants but also from fragments of large granules. The mixture of small and large granules, freed from the gelatinous material, is then subjected to repeated centrifuging to obtain the two types of starch.

of wheat starch granules with curves for the small and large granule preparations is given in Figure 6. These data indicate that the shape of the curve is not determined by the presence of a mixture of various-sized granules in the preparation but is dependent on differences in the character of the intermolecular bonds within the granule.

It should be emphasized that the steps or stages in gelatinization as indicated by the gelatinization curves are not sharp transitions occurring at one temperature, but each is a gradual transition covering a range of 10°–15°C, and may be further subdivided. An entire step or stage cannot be passed through by holding the temperature constant

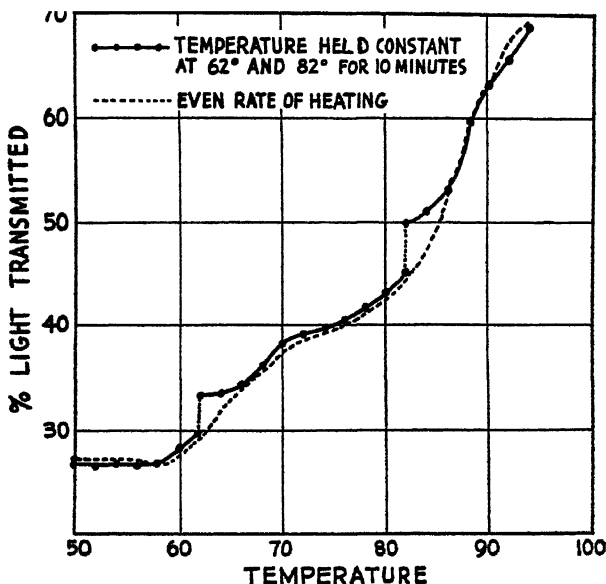


Fig. 7. Effect of holding the temperature constant for a period of time during the gelatinization procedure.

at the beginning of the step, e.g., the first step in the gelatinization of wheat starch occurring between the temperatures of 55°C and 70°C cannot be completed by holding the temperature constant at 55°, at 60°, or even at 65°. This is illustrated in Figure 7. However, it is to be noted that an increase in light transmission does occur on holding the temperature constant for a time. This indicates that the curve might be considerably altered by exceedingly slow heating, although, as before stated, the curves for wheat and corn starches were not changed by varying the rate of heating from one requiring less than 30 minutes to one requiring over 4½ hours for a curve complete from 30° to 95°.



### Summary

Equipment was developed for adapting the spectrophotometer to the study of starch gelatinization.

It was found that within the limits of from 2.5°C to 0.15°C per minute, the rate of heating had no effect on the curve characteristics nor on the gelatinization temperatures of wheat and corn starches.

The size of the starch granule determined the amount of light transmitted by ungelatinized starch suspensions. Large and small granule wheat starch preparations gave curves essentially similar in character, indicating that the two types of wheat starch granules are similar in gelatinization characteristics.

The data confirm the statement of Katz (1928) that gelatinization takes place in steps and that for some starches these steps occur at widely differing temperatures.

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**METHODS FOR DETERMINATION OF ALPHA-AMYLASE.**  
**IV. A GLASS END POINT COLOR STANDARD**  
**FOR USE IN THE DEXTRINIZING METHOD;**  
**EFFECT OF TEMPERATURE AND STARCH**  
**LOT ON THIS METHOD**

SUTTON REDFERN

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(Presented at the Annual Meeting, May 1946; received for publication May 5, 1947)

The Wohlgemuth procedure developed by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-amylase has achieved widespread popularity. The method promises to become even more widely used now that a commercial source of pure beta-amylase is available for preparing the substrate.

In this method, alpha-amylase activity is expressed in terms of the digestion time required for the enzyme to convert starch to products which give a red-brown coloration with iodine. The standard end point is the color produced by a specified combination of Merck's Reagent dextrin and iodine. This end point is an improvement both over the previously used erythropoint at which the color changes from blue to red, and over the achromic point at which the color disappears. However, no critical study of the dextrin-iodine end point color has been reported. It is one purpose of this paper to describe a new permanent glass color standard to replace the dextrin-iodine standard.

**Comparison of Different Lots of Dextrin**

Dextrins are degradation products of starch. It was thus expected that different lots of dextrin would vary with respect to their iodine color. To substantiate this, spectrophotometric data for five different lots of Merck's Reagent dextrin were obtained with a Beckman quartz spectrophotometer using 1 cm cells and a water reference blank. Transmittancy values were measured in 10 m $\mu$  steps. The dextrin-iodine color was prepared as described by Sandstedt, Kneen, and Blish using a weight of dextrin equivalent to 0.528 g dry dextrin per liter of water.

In Figure 1, the transmittancy values for each dextrin-iodine color are plotted against wavelengths of light. Because of the presence of excess iodine, all of the dextrin-iodine solutions completely absorbed all light below a 500 m $\mu$  wavelength. The curves show wide variations in the color produced by each dextrin. Moreover, the crossing of the curves for dextrins Nos. 1 and 2 even indicated differences in color

hues, since the solutions were of equal optical density, as will later be shown in Table I.

The curves in Figure 1 are not sufficient to define the quantitative effect on the dextrinizing method of the variations in dextrin lots. Therefore, each dextrin-iodine color was used as the end point for determining the dextrinizing time of a standard barley malt sample. Total light transmittance, expressed as optical density, was measured

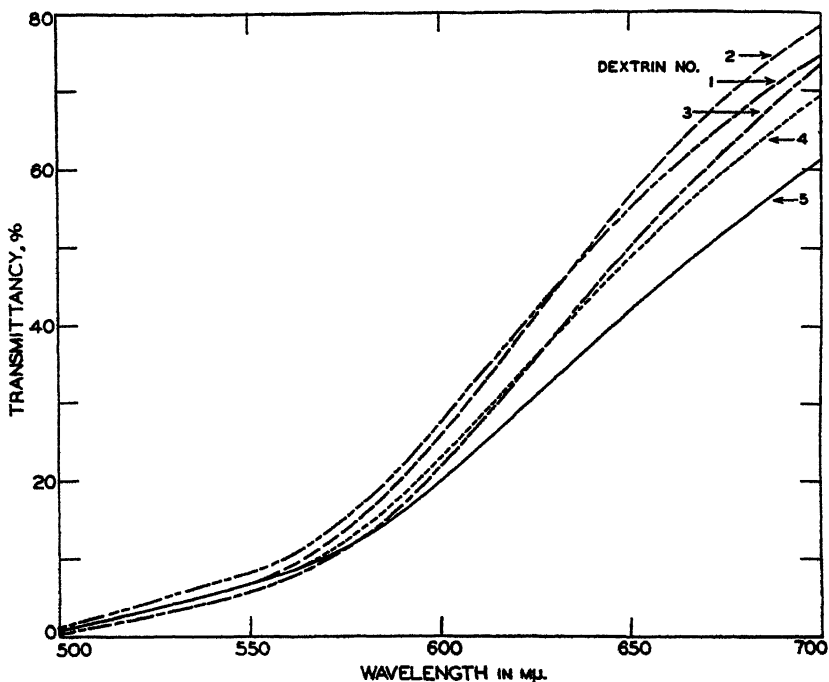


Fig. 1. Transmittancy curves for dextrin-iodine solutions.

with an Evelyn colorimeter without a filter. The hydrolysis rate of the beta-amylase starch substrate was followed by measuring the transmittancy of the hydrolyzate-iodine color at appropriate time intervals with the Evelyn colorimeter. By plotting transmittancy against reaction time, the dextrinizing time corresponding to the optical density of each dextrin-iodine solution was determined. The dextrinizing time was also determined visually using each dextrin-iodine color in a Hellige comparator.

The optical density of each dextrin-iodine color and the dextrinizing times for each as determined by the Evelyn colorimeter and the Hellige comparator are given in Table I. The dextrinizing times determined by the Evelyn colorimeter varied from 15.75 to 19.75 minutes,

a range of four minutes or about 22% of the average. In the Hellige comparator, the times varied from 17.25 to 19.0 minutes, a range of 1.75 minutes or about 10% of the average. Since it is easily possible to have duplicate analyses agree within 0.25 minute, the differences in dextrinizing times obtained with either method are much too large to be neglected.

TABLE I  
EFFECT OF DIFFERENT LOTS OF MERCK'S REAGENT DEXTRIN  
ON DEXTRINIZING TIME

Dextrin no.	Optical density	Dextrinizing time	
		Evelyn colorimeter	Hellige comparator
		<i>Min.</i>	<i>Min.</i>
1	0.463	19.75	19.0
2	0.464	19.75	18.5
3 <sup>1</sup>	0.511	18.25	18.0
4	0.517	18.0	18.0
5	0.600	15.75	17.25

<sup>1</sup> Sample used for 1946 collaborative study by Malt Evaluation Committee, A. A. C. C.

The differences between dextrinizing times as determined by the Evelyn colorimeter and by the visual comparator are due, most probably, to the differing response of the eye and the photocell, and to the fact that the hue of the dextrin-iodine color does not exactly match that of the hydrolyzate-iodine color at the end point.

The dextrin-iodine color standard also has other disadvantages. Neither stock dextrin nor dextrin-iodine solutions are stable. Kneen (1946) recommended that a fresh dextrin-iodine solution be prepared daily and a fresh dextrin stock solution monthly. It has also been this author's experience that the hue of the dextrin-iodine color is not the same as the hue of the hydrolyzate-iodine color, at least when they are compared at a solution depth of 13 mm. This makes it difficult to select the exact end point.

### Inorganic Color Standards

A permanent color standard would be very desirable. Redfern and Landis (1946) and Olson, Evans, and Dickson (1944) have described inorganic standards made from cobalt chloride, potassium dichromate, and hydrochloric acid, which differ only in the proportions of each component. Neither of these standards exactly matched the hue of the dextrin-iodine color. The solutions were not spectrophotometrically equivalent, so that the color match was only visual. This may be shown by comparing the transmittancy curve for the Landis-Redfern color standard in Figure 2 with the transmittancy

curves for the dextrin-iodine colors in Figure 1. It should be noted that visual match is dependent upon viewing depth, since both the inorganic color solutions and the dextrin-iodine solution are dichroic, i.e., different thicknesses exhibit different hues.

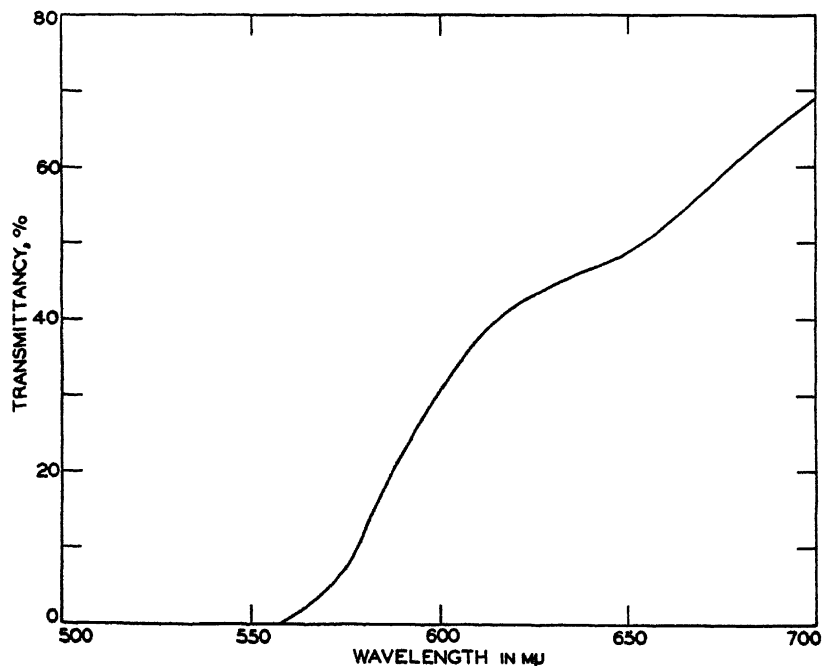


Fig. 2. Transmittancy curve for Landis-Redfern inorganic color standard.

In an attempt to find one combination of inorganic salts that was spectrophotometrically equivalent to the dextrin-iodine color, a study was made of various combinations of cobalt sulfate, cobalt chloride, ferric chloride, copper sulfate, potassium dichromate, uranyl acetate, and potassium chloroplatinate. No such universal combination was found. Each depth of the dextrin-iodine color required a different mixture of the inorganic salts.

#### Hellige Varnish Color Standard

A permanent glass color standard was sought, which would be non-fading, stable, and always ready for use. It was found that the No. 17 Hellige varnish color is a very close visual color match to the dextrin-iodine color at a depth of 13 mm. This varnish color is one of the 18 standard varnish colors of the Institute of Paint and Varnish Research, and, therefore, has the advantage of being available as a

stock item. A comparison of five different No. 17 varnish colors showed deviations between them no greater than 2%. This accuracy should be compared with the 25% difference shown in Table I between the optical density extremes of the iodine colors from different lots of dextrin.

The No. 17 varnish color has the further advantage of matching the hue of the hydrolyzate-iodine color much better than does the dextrin-iodine color. The importance of this factor is readily apparent.

### **Hellige Varnish Color Standard and Comparator Applied to Dextrinizing Method**

The No. 17 varnish color standard can be used in either the standard Hellige comparator (607)<sup>1</sup> or the pocket comparator with prism attachment (605-A).<sup>1</sup> The regular varnish color disc contains a series of colors from Nos. 11 to 18, but a special alpha-amylase color disc (620-S5)<sup>1</sup> containing only the No. 17 color may be obtained at a lower cost than the complete disc. This special disc is the preferred color standard because the manufacturing tolerance is much smaller than that permitted for the same color in the regular varnish color disc. The center of the complete varnish color disc normally contains a light green glass filter which must be removed. The special alpha-amylase color disc does not have this filter.

The comparator is conveniently illuminated with a 100-watt frosted bulb. Comparison of the hydrolyzate-iodine color with the No. 17 varnish color involves use of a square tube of 13 mm viewing depth which is standard equipment with the Hellige comparator. The square tube is recommended in preference to round test tubes which must be selected to have a 13 mm viewing depth.

The dextrinizing method makes use of the No. 17 varnish color and Hellige comparator as follows. The substrate and reagents are prepared according to the directions of Sandstedt, Kneen, and Blish (1939). A series of 13 × 100 mm test tubes containing 5 ml of dilute iodine solution "B" is prepared and attemperated at 30°C in readiness for testing. As the end point is approached, 1 ml of the hydrolyzing mixture is added to 5 ml of iodine solution and the hydrolyzate-iodine solution is poured into the 13 mm square tube for color comparison with the No. 17 varnish color. After the color comparison is made, the solution is poured out by giving the tube a quick shake. In this way, very little liquid remains in the tube and it is ready for another test. It is the author's custom to remove a sample every 0.5 minute on the minute or half-minute. In case two samples 0.5 minute apart show

<sup>1</sup> Catalog numbers of Hellige, Inc., Long Island City, New York, N. Y.

that one is darker and the other lighter than the No. 17 color standard, the dextrinizing time is interpolated as the quarter minute between these two times.

Since the dextrin-iodine color standard varies with the dextrin batch, it is proposed that the No. 17 varnish color be accepted as the standard end point color when the hydrolyzate-iodine color is viewed in a 13 mm square tube.

Kneen and Sandstedt (1941) published a method for the determination of beta-amylase in which the saccharification due to the alpha-amylase was subtracted from the total saccharifying activity. A table relating alpha-dextrinogenic activity to alpha-saccharogenic activity was compiled, but this table is only valid for alpha-amylase activities determined with the aid of the particular dextrin sample used by these authors. It cannot be used by other workers using a different dextrin without introducing a possible error. The magnitude of this error has not as yet been determined, but it will be necessary to re-examine the relationship between alpha-dextrinogenic and alpha-saccharogenic activity using the new color standard. This same consideration applies to the work of Olson, Evans, and Dickson (1944).

### Effect of Temperature on Iodine Colors

Although it is a well-known fact that the familiar blue starch-iodine color disappears when heat is applied (Kerr, 1944), the effect of temperature on the hydrolyzate-iodine and dextrin-iodine colors has not previously been reported. For the determination of temperature effect, a dextrin-iodine color standard using dextrin No. 3, and a hydrolyzate-iodine color which corresponded approximately to the end point were prepared. Each solution was adjusted to the desired temperature, and the total transmittancy was measured with an Evelyn colorimeter without a filter. The results are shown in Figure 3. Surprisingly, the dextrin-iodine color is nearly independent of temperature in the range from 15° to 30°C. The hydrolyzate-iodine color, on the contrary, has a larger temperature coefficient. The difference in optical densities at 20° and 30°C amounts to approximately 8%. In practice this temperature effect cannot be neglected.

The actual effect of temperature on dextrinizing time was determined by running duplicate hydrolyses using the same quantity of barley malt and the same substrate at 30°C. In one case the iodine solution was attemperated at 20°C and in the other at 30°C. The dextrinizing times for each temperature using the No. 17 varnish color as an end point are given in Table II.

The dextrinizing times differ by nearly 10% and this cannot be viewed with equanimity. The temperature of the hydrolyzate-iodine

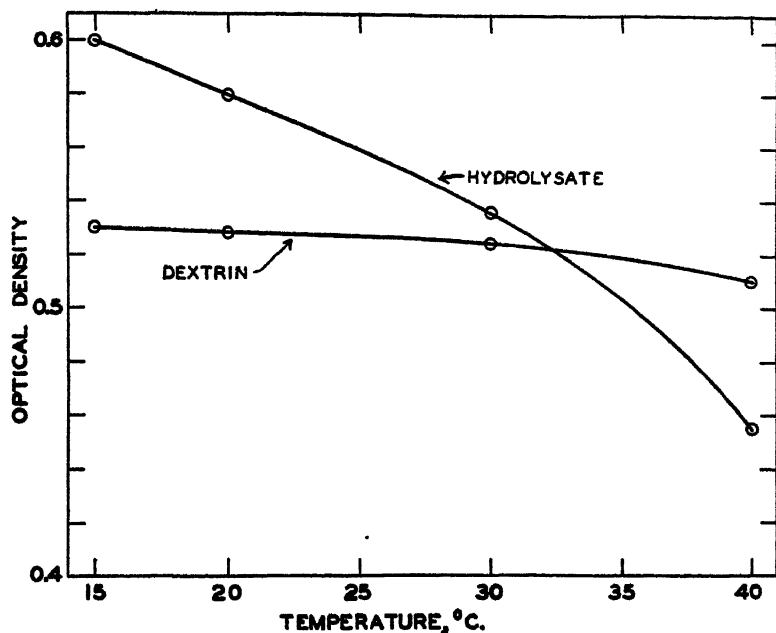


Fig. 3. Effect of temperature on dextrin-iodine and hydrolyzate-iodine solutions.

TABLE II  
EFFECT OF TEMPERATURE OF IODINE SOLUTION  
ON DEXTRINIZING TIME

Temperature of iodine solution	Dextrinizing time	Alpha-amylase units (SKB)
°C	Min.	
20	18.75	32.0
30	17.0	35.3

color must be controlled. This is most easily done by attemperating the test tubes containing the iodine solution in the same 30°C water bath used for the digestion. It is true that the temperature may change slightly during the time required to make the color comparison, but this change will be practically constant.

#### Effect of Starch on Dextrinizing Method

For the preparation of the beta-amylase starch substrate, Sandstedt, Kneen, and Blish (1939) originally specified Merck's soluble Lintner starch. This was later changed to Merck's soluble Lintner starch, special for diastatic power determination (Kneen, 1946). The latter is the one approved by the American Society of Brewing Chemists for use in the determination of diastatic power. However, the



A. S. B. C. (1944) also approved the use of the soluble Lintner starch made by the J. T. Baker Chemical Co. Several different lots of soluble starch from each of the two companies were compared by preparing a substrate from each starch using Wallerstein beta-amylase and determining the dextrinizing time of a standard quantity of malt syrup acting on these substrates. The results in terms of alpha-amylase units are given in Table III.

TABLE III  
EFFECT OF DIFFERENT LOTS OF SOLUBLE STARCH  
ON THE DEXTRINIZING METHOD

Description of soluble starch	Alpha-amylase units
Merck's lot no. 40515	75.4
40455	75.7
71741	76.0
111243 <sup>1</sup>	76.0
111243 <sup>1</sup> Different bottles	76.8
11446 <sup>1</sup>	78.4
4445 <sup>1</sup>	78.8
Baker's lot no. 7344	80.5
51045	81.8
32743	81.8
72045 <sup>1</sup>	82.7
102142	85.0
121043	85.4

<sup>1</sup> Approved by the American Society of Brewing Chemists for diastatic power determination.

Merck and Baker starches fall into two distinct groups, with the Baker starches giving consistently higher values. The number of alpha-amylase units (SKB) for Merck starches varies from 75.4 to 78.8 with an average value of 76.7 units, and the Baker starches range from 80.5 to 85.4 with an average value of 82.9 units. Thus, the extreme variation of all the starches tested amounted to 10.0 alpha-amylase units (SKB), or approximately 12.5%. The average values for the approved Merck and Baker starches differed by about 5%. This is a definite difference which cannot be ignored.

Differences in starch lots are not unexpected and should not be construed as a criticism of either manufacturer. Approval of certain of these starches applies only for use in the official diastatic power method; they were not standardized for use in the dextrinizing method. However, there is evidently a fundamental difference in the preparation of these starches by each manufacturer and it is unfortunate that the starches are not interchangeable for use with the two methods. Hence, it is probable that the starch will have to be standardized for use in the dextrinizing method the same as is done for the diastatic power method, though it would be preferable to have one starch that could be used for both methods.

### Summary

The effect of different lots of Merck's Reagent dextrin on the dextrin-iodine end point color standard used in the method of Sandstedt, Kneen, and Blish for the determination of alpha-amylase was studied. Different dextrans gave different spectrophotometric curves and when used as end points resulted in dextrinizing time differences ranging from about 10 to 20%.

It was found that the No. 17 Hellige glass varnish color standard when used with a Hellige comparator can replace the dextrin-iodine color standard with considerable advantage. The glass standard is permanent, reproducible, commercially available, and independent of temperature. The application of the varnish color standard to the dextrinizing method is herein described. This new glass standard is proposed as a replacement for the dextrin-iodine standard.

The dextrin-iodine color was found to be nearly independent of temperature, but the hydrolyzate-iodine color varies considerably with temperature. The dextrinizing time of a malt was significantly shorter when the temperature of the iodine solution was 30°C than when the temperature was 20°C. It is recommended that the dilute iodine solution be attemperated at 30°C before use to insure a standard temperature of testing.

Different lots of Lintner soluble starch showed a maximum variation of approximately 12.5% in alpha-amylase values obtained with the same malt, although starches approved for the diastatic power method differed by approximately 5%. There is a significant difference between soluble starches made by Merck and by Baker, the Baker starches giving higher alpha-amylase values.

### Acknowledgment

Grateful acknowledgment is made to Dr. Allan D. Dickson for supplying samples of different lots of soluble starch, to the Merck Company for supplying known samples of Reagent Dextrin, and to the J. T. Baker Chemical Company for supplying samples of soluble starch.

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## B-COMPLEX VITAMINS IN GRAIN SORGHUMS

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Grain sorghums are grown extensively in those areas of the Southwest where climatic conditions make corn production hazardous. Because of their ability to withstand high-temperature and low-moisture conditions, they have become an important crop in Texas, Kansas, Oklahoma, California, New Mexico, Nebraska, and Colorado.

During the decade 1930-1939, production of grain sorghums in the United States averaged about 52 million bushels annually. Owing to the development of dwarf types of sorghum suitable for machine harvesting and adapted to climatic conditions in this country, production has increased greatly. In 1944 about 182 million bushels were harvested from an estimated 9.1 million acres. Almost an equivalent acreage was planted for forage and silage.

Information concerning the production, composition, and uses of sorghums has been compiled by Edwards and Curtis (1943). As with corn, approximately 85% of the sorghum crop is used on farms. In view of this extensive use for feeding purposes and the limited data at hand concerning the vitamin content of this cereal, further information seemed desirable. Such data should allow better estimation of the vitamin contents of some derivatives and by-products resulting from the use of sorghums in industries such as distilling and milling.

The vitamin content of sorghums has received little attention. By a rat-growth method, yellow corn was reported (Smith, 1930) to have about 10 times as much vitamin A activity as yellow milo sorghum and about 20 times as much as the Hegari variety. Nordgren and Andrews (1941) reported that thiamine in seven unidentified sorghums varied from 1.93 to 3.97 mg per pound and averaged 2.68 mg per pound

<sup>1</sup> One of the laboratories of the Bureau of Agriculture and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(4.75, 8.75, and 5.9  $\mu\text{g}$  per gram respectively). Riboflavin, nicotinic acid, and pantothenic acid contents of 29 varieties of grain sorghum grown in 1941 and 1942 at Perkins and Woodward, Oklahoma, have been reported by Knox *et al.* (1944). Riboflavin varied from 1.17 to 2.08  $\mu\text{g}$  per gram, pantothenic acid from 12 to 15  $\mu\text{g}$  per gram, and nicotinic acid from 22 to 78  $\mu\text{g}$  per gram.

In this report values are given for riboflavin, nicotinic acid, pantothenic acid, pyridoxine, and biotin contents of 42 sorghum strains. Samples were obtained from several locations through the cooperation of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

### Methods

All vitamin analyses of finely ground sorghum grain were made by microbiological methods. Riboflavin was determined by the Snell and Strong method (1939) following hydrolysis of the sample in 0.1 *N* hydrochloric acid for 30 minutes at 120°C, filtration after adjustment to pH 4.5, and, finally, neutralization to pH 6.8. Nicotinic acid determinations were by the method of Snell and Wright (1941) after hydrolysis of samples in 1 *N* sulfuric acid for 30 minutes at 120°C and filtration after neutralization to pH 6.8. Pantothenic acid was determined with *Lactobacillus arabinosus* (Skeggs and Wright, 1944). For this determination samples were steamed for 15 minutes, cooled, and digested for 16 to 20 hours at 37°C with a mixture of papain and clarase suspended in an acetate buffer, pH 4.6. Biotin and pyridoxine were liberated by autoclaving at 120°C for 30 minutes in the presence of 5 *N* sulfuric acid; biotin was determined in the neutralized filtrates with *L. arabinosus* (Wright and Skeggs, 1944). The same filtrates were assayed for pyridoxine with *Saccharomyces cerevisiae* (Atkin *et al.*, 1943). Moisture content was determined by drying 2-g samples at 130°C for 1.5 hours in a forced-draft oven.

### Results

The results of the analyses of grain sorghums are contained in Table I. Waxy and nonwaxy varieties show essentially the same variations. Thus there seems to be no correlation between vitamin content and the type of starch stored in the grain. The riboflavin content is only slightly higher than that of yellow hybrid corn. Assays ranged from 0.81 to 2.03  $\mu\text{g}$  per gram, with an average of about 1.3  $\mu\text{g}$ . On the other hand, grain sorghums are 2.5- to 3-fold richer than corn in biotin. Two samples, a waxy kafir (T.S. 25289) from Lubbock, Texas, and Midland from Hays, Kansas, in 1944 were low in comparison with other sorghums, yet contained quantities of biotin equal to

TABLE I  
VITAMIN CONTENT OF GRAIN SORGHUMS

Variety	Crop year	Place grown	Vitamin content—moisture-free basis				
			Riboflavin $\mu\text{g/g}$	Nicotinic acid $\mu\text{g/g}$	Pantothenic acid $\mu\text{g/g}$	Biotin $\mu\text{g/g}$	Pyridoxine $\mu\text{g/g}$
<i>Waxy sorghums</i>							
Sagrain	1939	Stoneville, Miss.	0.95	27.7	4.6	0.28	2.8
Leoti	1941	Lincoln, Nebr.	.81	37.6	7.4	.23	3.5
Leoti	1942	Hays, Kansas	1.31	34.1	9.9	.28	4.0
Cody	1942	Hays, Kansas	.96	72.1	9.8	.24	5.1
Cody	1943	Hays, Kansas	.96	72.9	7.6	.24	4.8
Leoti X Atlas, H.C. 40-17	1943	Hays, Kansas	1.41	50.2	8.5	.30	7.3
Leoti X Atlas, H.C. 41-13	1943	Hays, Kansas	—	54.0	8.9	.28	8.6
Waxy kafir, T.S. 25289	1943	Lubbock, Texas	1.32	53.1	9.7	.09	7.9
Waxy milo, S.A. 5404-1	1943	Lubbock, Texas	1.56	55.9	8.1	.21	6.6
Waxy Leoti X 71-2-13-9	1943	Woodward, Okla.	.84	43.8	9.9	.11	5.1
Texas Waxy kafir	1943	Lincoln, Nebr.	1.26	58.7	10.3	.28	7.9
Cody	1944	Hays, Kansas	1.43	70.5	8.7	.24	6.8
Leoti X Atlas, H.C. 42-27	1944	Hays, Kansas	.96	48.4	9.2	.27	6.8
Cody X Wonder Club, H.C. 44-2045	1945	Hays, Kansas	1.41	83.1	8.6	.32	7.1
Cody X Wonder Club, H.C. 45-112	1945	Hays, Kansas	1.59	91.9	8.9	.30	4.8
Cody X Wonder Club, H.C. 45-140	1945	Hays, Kansas	1.58	83.2	7.8	.34	4.6
Yellow Waxy hybrid, H.C. 45-5173	1945	Hays, Kansas	1.50	78.8	7.9	.36	4.7
Yellow Waxy hybrid, H.C. 45-5177	1945	Hays, Kansas	1.68	71.4	7.4	.33	4.7
Cody	1945	Hays, Kansas	1.29	71.8	8.7	.21	4.2
Waxy Atlas, H.C. 41-20	1945	Hays, Kansas	1.39	45.5	7.8	.35	5.2
Leoti X Atlas, H.C. 42-29	1945	Hays, Kansas	.90	50.5	10.2	.30	5.3
Leoti	1945	Hays, Kansas	1.74	36.3	12.0	.46	6.3

TABLE I—Continued

Variety	Crop year	Place grown	Vitamin content—moisture-free basis				
			Riboflavin µg/g	Nicotinic acid µg/g	Pantothenic acid µg/g	Biotin µg/g	Pyridoxine µg/g
<i>Nonvasey sorghums</i>							
Atlas	1942	Hays, Kansas	1.11	35.7	5.9	0.23	4.8
Wonder Club	1942	Hays, Kansas	.65	65.2	6.9	.22	5.2
Kalo	1943	Hays, Kansas	1.59	43.4	5.6	.28	6.0
Early Kalo	1943	Hays, Kansas	1.06	31.4	5.6	.29	5.4
Wonder Club	1943	Hays, Kansas	1.22	70.0	6.1	.18	8.4
Texas Blackhull kafir, T.S. 9195	1943	Lubbock, Texas	1.11	33.7	5.2	.32	7.3
Texas milo, T.S. 25243	1943	Lubbock, Texas	2.03	66.2	8.7	—	—
Club kafir	1944	Woodward, Okla.	.90	56.7	6.3	.25	8.0
Midland	1944	Hays, Kansas	1.69	45.9	5.8	.09	7.0
Club kafir	1945	Hays, Kansas	1.11	45.4	7.5	.36	6.4
Club X Day, H.C. 40-431	1945	Hays, Kansas	.93	54.7	10.6	.39	6.5
(Leoti X Club) X Custer, H.C. 44-115	1945	Hays, Kansas	1.32	38.3	6.1	.38	6.9
Midland X Wonder Club, H.C. 44-2112	1945	Hays, Kansas	1.24	57.3	5.8	.35	6.2
Midland X Wonder Club, H.C. 44-2117	1945	Hays, Kansas	1.54	72.6	7.6	.40	7.5
Cody X Wonder Club, H.C. 45-110	1945	Hays, Kansas	1.53	76.6	6.2	.37	4.7
Martin	1945	Hays, Kansas	1.46	34.3	10.3	.36	6.5
Westland	1945	Hays, Kansas	1.75	49.1	11.7	.39	7.5
Midland	1945	Hays, Kansas	1.32	48.9	4.6	.29	5.3
White durra	1945	Davis, Calif.	.82	53.9	9.8	.34	2.1
Double Dwarf Yellow milo	1945	Davis, Calif.	1.00	73.9	14.8	.38	2.5

that found in corn. While variation is noted in the pyridoxine levels, most samples contained only slightly less than corn.

Pantothenic acid levels ranged from 4.6 to 14.8  $\mu\text{g}$  per gram. Analyzing other varieties, Knox *et al.* (1944) reported values of approximately 12 to 15  $\mu\text{g}$  per gram.

Grain sorghums are notably richer than corn in nicotinic acid. Furthermore wide variation exists in the several varieties reported. Values range from 27.7  $\mu\text{g}$  per gram for Sagrain to 91.9  $\mu\text{g}$  per gram for a Cody  $\times$  Wonder Club selection (H.C. 45-112). Similar differences were reported by Knox *et al.* (1944).

### Discussion

The wide differences found in nicotinic acid levels suggested that by planned hybridization the content of this vitamin may be increased over that found in present commercial varieties. Several examples support this hypothesis. The Cody variety, now of particular interest because of its waxy starch, represents a selection from a Leoti  $\times$  Club cross. The three Leoti samples which were analyzed contained 37.6, 34.1, and 36.3  $\mu\text{g}$  per gram of nicotinic acid, while two Club Kafir samples contained 56.7 and 45.4  $\mu\text{g}$  per gram. Yet Cody of the 1942-1945 crop years contained 70.5 to 72.1  $\mu\text{g}$  per gram. Further evidence supporting this view is contained in the data for the Leoti  $\times$  Atlas crosses. The Atlas sample contained 35.7  $\mu\text{g}$  per gram, approximately the same level as found in Leoti. Strains of the cross that were tested contained 48.4 to 54.0  $\mu\text{g}$  of nicotinic acid per gram. Two yellow waxy hybrids of unknown parentage (H.C. 54-5173 and H.C. 45-5177) are of interest also, since they contained 78.8 and 71.4  $\mu\text{g}$  per gram of this vitamin.

A nonwaxy hybrid, Wonder Club, contained 65.2  $\mu\text{g}$  of nicotinic acid per gram. This variety is thought to be a cross of kafir  $\times$  feterita. Knox *et al.* (1944) reported a series of standard kafir strains to contain 28.8 to 33.6  $\mu\text{g}$  per gram (different crop years and locations). Somewhat higher values are reported in this paper for kafir types, ranging from 33.7 to 56.7  $\mu\text{g}$  per gram. Feterita strains are reported to contain nicotinic acid in the range of 43.7 to 56.2  $\mu\text{g}$  per gram (Knox *et al.*, 1944).

Furthermore, crosses involving varieties such as Cody and Wonder Club, both of which contain unusually large amounts of nicotinic acid, resulted in progeny of which some contained even more nicotinic acid. Three waxy selections are reported, containing 83.1 to 91.1  $\mu\text{g}$  of nicotinic acid per gram, while a nonwaxy selection contained 76.6  $\mu\text{g}$ . These differences may be attributable to chance selection.

Though some variation occurs in the levels of the other vitamins, and though wide differences between maximum and minimum values for pantothenic acid and pyridoxine contents are reported, the evidence does not suggest that the contents of B-complex vitamins other than nicotinic acid can be altered appreciably by hybridization. The pyridoxine contents of the two California-grown varieties, White durra and Double Dwarf Yellow milo, as well as that of the Sagrain from Mississippi, are substantially lower than those found in other varieties from elsewhere. Cody sorghum grown at Hays, Kansas, varied in content from year to year as follows: 1942, 5.1  $\mu\text{g}$  per gram; 1943, 4.8; 1944, 6.8; 1945, 4.2. In general, variations in riboflavin contents were not appreciable, though seven samples contained less than 1.0  $\mu\text{g}$  per gram. Such variation in vitamin levels may be the result of variations in soil fertility, climatic conditions, and other factors.

### Summary

Analyses are presented for the riboflavin, nicotinic acid, pantothenic acid, biotin, and pyridoxine contents of 42 samples of grain sorghums. Compared to corn, they contain approximately the same quantities of riboflavin and pyridoxine and more pantothenic acid, nicotinic acid, and biotin.

The data suggest that nicotinic acid contents may be significantly increased through hybridization. The Cody variety (70 to 72  $\mu\text{g}$  per gram) represents a cross of Leoti (approximately 35  $\mu\text{g}$  per gram) and Club (approximately 45  $\mu\text{g}$  per gram). Further increases, to as high as 91.9  $\mu\text{g}$  per gram, were found in Cody  $\times$  Wonder Club strains.

### Acknowledgment

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## IS THE DEGREE OF GRITTIENESS OF WHEAT FLOUR MAINLY A VARIETAL CHARACTER?

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A property of wheat flour that is of great practical importance is its structure or grittiness, i.e., its greater or lesser tendency to form into lumps. Flour that forms lumps is more difficult to use than flour that pours almost as smoothly as fine dry sand. The nonlumping type is easy to work up into a homogeneous mixture, whether for baking, sauces, or thickenings.

It is no doubt rather generally assumed that grittiness depends on protein content. Some support for this opinion is found in the fact that Manitoba spring wheat of high protein content mills into a flour possessing great grittiness, whereas the improved winter varieties of northern Europe with their generally low protein content yield a flour of unsatisfactory grittiness. As will be shown below, there is no valid evidence for the accuracy of this assumption.

In the wheat-breeding work at Weibullsholm attention has been directed to possible genetic factors, independent of the crude-protein content, that might affect the grittiness in flour. The first observations pointing in that direction were made some 10 years ago when a number of new winter wheat crosses with the Hungarian variety Bánkuter 178 as a parent were test milled for baking studies. Among these new varieties it was astonishing to find some which, while remarkably easy to mill, gave a flour that possessed a high degree of grittiness, deviating widely from that of the common Swedish winter varieties. In the course of years a number of such varieties were found and examined, but the majority were rejected because of unsatisfactory agronomic qualities. One variety possessing grittiness,

however, was made available to agriculturalists in 1943. It is Weibulls' Eroica wheat, which was raised from the cross (Bänkuter 178 × Standard) × Åring. Thanks to its combination of such valuable characters as relatively good winter-hardiness, very stiff straw and—compared with its rivals—very superior grain-yield, it has now attained a dominating distribution in the plains of southern Sweden. Under identical nitrogen-manuring conditions, Eroica wheat has a somewhat lower protein content than other Swedish winter varieties of wheat. Accordingly, it is remarkable that Eroica should deviate so much from its competitors in respect to grittiness. That this variety belongs to a distinct class is immediately evident from visual inspection of poured-out flour samples, and this has been fully confirmed in the sifting tests reported in this paper.

### Methods

The material was milled on a Buhler laboratory mill having a 0-60 % extraction capacity. For every test 100 g of flour were used, and sifting continued for 30 minutes. A shaker type of sifter was used, driven at a speed of about 340 strokes per minute. The recorded values are the means of three different tests, thus allowing of a statistical treatment of the material.

### Results and Discussion

*Winter Wheat Trials.* Table I shows the results of yield trials for a series of winter wheat varieties grown under uniform conditions. The

TABLE I  
RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT,  
AND GRITTIENESS OF FLOUR  
(Winter Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Grittiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
Standard	8.6	2.8	16.2	81.0
Standard II	8.9	4.6	15.0	80.4
Åring II	9.4	3.4	15.2	81.4
Åring III	9.0	3.7	15.2	81.1
Eroica	8.4	7.0	27.8	65.2
Skandia II	8.9	3.8	16.2	80.0
Skandia III	8.6	4.0	15.5	80.5
Borg	9.1	4.2	17.1	78.7
Standard error <sup>1</sup>		0.44	1.52	1.92

<sup>1</sup> Applies to mean values for three determinations.

protein content of the flour was lowest in the Eroica wheat, 8.4%, and highest in Åring wheat II, 9.4%. Grittiness data show that in spite of having the lowest protein content Eroica wheat occupies an absolutely distinct position; only 65.2% of the flour passes through cloth 15, while the corresponding percentages for the other varieties lie between 78.7 and 81.4%. The difference between the last two values is not statistically significant. On the other hand, the considerably divergent figure for Eroica wheat must be regarded as a significant expression of the exceptional position occupied by that variety.

TABLE II  
RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT,  
AND GRITTIENESS OF FLOUR  
(Winter Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Grittiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
New variety out of Eroica × Virtus	8.8	4.7	19.2	76.1
Virtus	8.0	3.8	13.3	82.9
Ergo	8.1	4.3	17.7	78.0
Eroica	7.7	6.8	27.2	66.0
New variety from Eroica × Virtus	7.8	8.6	26.0	65.4
New variety from Eroica × Virtus	8.3	5.9	25.8	68.3
Standard error <sup>1</sup>		0.73	2.28	2.93

<sup>1</sup> Applies to mean values for three determinations.

A second trial with winter wheat was made with Ergo, Eroica, and Virtus, and with three new varieties obtained from the cross between Eroica and Virtus. Eroica has a high grittiness, while Virtus is not satisfactory in this respect. The results of this second trial are given in Table II. Eroica again shows the lowest protein content, 7.7%, while the highest figure is 8.8%. In grittiness, two of the varieties raised from the cross Eroica × Virtus are fully equal to the mother variety Eroica. The third is somewhat better than the paternal variety Virtus, but both these varieties and Ergo must be assigned to the class possessing unsatisfactory grittiness.

The data given in Tables I and II show no connection whatever between crude protein content and degree of grittiness. The high grittiness that is characteristic of Eroica wheat as well as of two of the progenies of Eroica × Virtus must be due to other hereditary factors,

which have apparently admitted of being rather freely recombined at crosses.

*Spring Wheat Trials.* Results from a variety trial of spring wheats are presented in Table III. By comparison with the winter varieties (Tables I and II), all spring varieties, except Fylgia, show rather high to very high grittiness. Fylgia wheat seems to belong to the unsatisfactory class, as 81.7% of the flour passed through cloth 15. Corresponding values for other varieties ranged from 61.1 to 69.2%. A statistically significant difference exists between the value for Fylgia and those for other varieties, and there are also some other significant differences. The decidedly divergent type of grittiness possessed by Fylgia wheat would thus seem immediately clear.

TABLE III  
RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT,  
AND GRITTIENESS OF FLOUR  
(Spring Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Gruttiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
Atle	10.1	5.5	26.8	67.7
Progress	9.7	7.2	31.7	61.1
Brons	10.6	6.3	28.3	65.4
Kärn	10.5	5.6	25.2	69.2
Diamant II	10.5	9.0	24.6	66.4
Fylgia	10.0	3.8	14.5	81.7
New variety from 8388 × 8473	9.4	6.3	26.8	66.9
New variety from 4099 × C 1833	8.9	6.6	26.7	66.7
Standard error <sup>1</sup>		0.99	1.76	2.12

<sup>1</sup> Applies to mean values for three determinations.

Comparison of the grittiness of the different varieties with their respective protein contents again provides no support for the assumption that the property of grittiness depends on the protein content. A notable fact is that the two good new varieties listed in Table III gave much higher grain yields than the market varieties, and accordingly show a relatively low crude-protein content, but are nevertheless relatively gritty. Another interesting point is that Brons wheat and Fylgia wheat—raised from the crosses Aurore × Extra Kolben, respectively, and therefore very closely related—are very different in grittiness though differing in protein content by only 0.6%. Possibly Fylgia inherited its unsatisfactory grittiness from its mother Aurore,

while the high degree possessed by Brons was transmitted from the father variety Extra Kolben II.

*Manuring Trials.* The dominant part that the variety, rather than the protein content, plays in grittiness is also illustrated by the results of nitrogen-manuring trials at Weibullsholm. The same wheat variety was supplied with different quantities of nitrogenous fertilizer—some applied as late as during the period of earing—and considerable differences in protein content were thus obtained. Had there been any correlation between protein content and grittiness, this would have manifested itself in the results from these nitrogen-manuring trials.

TABLE IV  
EFFECT OF NITROGEN FERTILIZATION ON PROTEIN CONTENT  
AND GRITTIENESS  
(Winter Wheat Variety Standard II, 1945)

Calcium nitrate added kg/hectare			Protein (d.m. basis)	Grittiness		
Date of application				Above		Through cloth 15
3/5	22/6	26/6		Cloth 12	Cloth 15	
			%	%	%	%
			7.3	4.0	15.5	80.5
200			7.7	4.0	17.0	79.0
350			8.6	4.3	16.2	79.5
200	150		8.9	4.5	18.3	77.2
500			9.1	4.2	17.0	78.8
200	300		9.7	4.1	18.0	77.9
800			11.0	4.5	16.4	79.1
200	300	300	11.8	3.5	14.5	82.0
Standard error <sup>1</sup>				0.12	0.44	0.52

<sup>1</sup> Applies to mean values for three determinations.

The results of tests undertaken on materials from a nitrogen-manuring trial of this kind with the winter wheat variety Standard II and with the spring variety Diamant II are recorded in Tables IV and V. The former shows protein contents rising evenly from 7.3 to 11.8%. The data for the degree of grittiness show only minor differences (though some are statistically significant) between different sections of the trial, but no correlation with the crude-protein content is discernible. Indeed, the least satisfactory figures for grittiness were obtained in the section with the highest crude-protein content. Table V, like Table IV, offers no evidence of any correlation between grittiness and crude-protein content in Diamant II. It is true that there are statistically significant differences, but these are not attributable to the difference in protein content but probably to the time at which

the nitrogenous fertilizer was applied. A somewhat surprising feature is that the trial-sections with respectively the lowest and next lowest crude-protein contents show the best grittiness figures.

The results of these two fertilizer trials, together with those previously cited from investigations on materials of different varieties, have given a clear verdict that it is primarily the variety and not the crude-protein content which decides the degree of grittiness possessed by wheat flour. Whether other cultivation factors, such as soil and type of year, exercise any influence must here be passed over, there being as yet no material available for the elucidation of this point.

TABLE V  
EFFECT OF NITROGEN FERTILIZATION ON PROTEIN CONTENT  
AND GRITTIENESS  
(Spring Wheat Variety Diamant II, 1945)

Calcium cyanide applied before showing	Calcium nitrate			Protein (d.m. basis)	Grittiness		
					Above		Through cloth 15
	3/5	19/6	25/6		Cloth 12	Cloth 15	
kg/hectare	kg/hectare	kg/hectare	kg/hectare	%	%	%	%
100				8.9	6.5	28.9	64.6
100	100			8.5	6.1	29.3	64.6
100	50	50		9.4	5.3	24.7	70.0
100	400			9.8	5.0	26.4	68.6
100	100	300		11.9	5.6	29.3	65.1
100	700			12.6	5.1	25.6	69.3
100	100	300	300	15.5	6.5	26.0	67.5
Standard error <sup>1</sup>					0.24	0.73	0.83

<sup>1</sup> Applies to mean values for three determinations.

*Relation between Grittiness and Vitreous Kernels.* According to the present writer's experience, it appears that wheat varieties that combine a low protein content with a high degree of grittiness contain a higher percentage of vitreous or semivitreous kernels than varieties with an equally high protein content and an unsatisfactory degree of grittiness. Thus, the vitreousness is not considered to depend solely on the protein content, an assumption that is confirmed by investigations made by Mr. Widebäck.<sup>1</sup>

Table VI presents the results of a study of the vitreousness and the protein content of two varieties with an unsatisfactory grittiness, Virtus and Standard, and of two varieties of satisfactory grittiness, Eroica and a new variety designated W:s 11376. The last-mentioned

<sup>1</sup> Mr. I. Widebäck has made quality tests for the Weibullsholm Institute for many years, and his permission to present the following results is gratefully acknowledged.

variety has been shown by sifting tests to belong to the same good class of grittiness as Eroica wheat. The materials for these tests were also grown under comparable conditions of soil, manuring, and so on.

The data show that Eroica and 11376, with their high degree of grittiness, have a decidedly greater tendency to vitreousness than the varieties Virtus and Standard. This is especially remarkable in the case of Eroica wheat, which had an average crude-protein content of only 7.8%, as against 8.2–8.3% in the other varieties. Hence the figures confirm the fact that vitreosity can to some extent depend on

TABLE VI  
RELATIONSHIP BETWEEN VITREOUSNESS, CRUDE-PROTEIN CONTENT,  
AND SIZE OF THE STARCH GRANULES

Variable	Virtus		Standard		Eroica		W : s 11376	
	Kernels	Protein	Kernels	Protein	Kernels	Protein	Kernels	Protein
Kernel	%	%	%	%	%	%	%	%
Vitreous	4.8	11.0	3.2	10.8	8.0	10.4	12.6	10.3
Semivitreous	19.0	8.5	16.0	8.9	26.0	8.1	22.2	8.6
Floury	76.2	7.9	80.8	7.9	66.0	7.3	65.2	7.7
Mean protein content		8.2		8.2		7.8		8.3
Starch granules, % smaller than 0.02 mm		64.0		54.0		71.0		69.0
Grittiness	Un-satisfactory		Un-satisfactory		Very satisfactory		Very satisfactory	

other factors than the crude-protein content, factors linked up with the genetically controlled properties of the variety. If this specific vitreousness, which does not depend on the vitreousness caused by an increasing protein content, exercises an influence upon the grittiness, the next question will be from what source the former kind of vitreousness originates.

The problem of the vitreosity has already been made the object of tests by several investigators. In the report of his results Mr. Widebäck refers to the following authors: Neumann (1929), Walldén (1923), and Hoffmann-Mohs (1931). Müller (1942) presents a short account of the vitreousness and its causes, a translation of which runs as follows: "On being soaked and sectioned the air-dried barley and wheat grains called 'melede' (floury) exhibit a snow-white, flutelike endosperm that is porous and friable. The grains are called 'glassede' (vitreous) when the endosperm is hard, firm, transparent and exhibits a more or less dark section-surface. Between these types there are regular transitions. The more air vacuoles the more floury is the endosperm; in the vitreous endosperm there are no air vacuoles. The vitreous

endosperm contains in solitary parts of the grain somewhat larger amounts of small starch granules than the floury type."

The four varieties mentioned above were analyzed as to their content of starch granules of smaller size than 0.02 mm by Mr. Widebäck. The results of these tests are given in the last column of Table VI. Eroica and W:s 11376 contained respectively 71 and 69% of starch granules smaller than 0.02 mm, as against 64 and 54% for Virtus and Standard. Even though investigations on a larger series are desirable to establish more reliable means, the results of the present analyses provide an acceptable explanation of the higher vitreousness in the varieties Eroica and W:s 11376 as compared with Virtus and Standard. The cause may well be simply that the comparatively large amounts of small starch granules contained in the two former varieties, together with the protein substance present, constitute a more compact endosperm and in that way prevent refractive air interspaces from being formed; while in the two latter varieties, with their larger starch granules, the mass is less compact and consequently more likely to contain air vacuoles, which makes the kernel more floury.

There is a possibility that the degree of grittiness is correlated with the vitreousness thought to be specifically linked to the variety and to owe its origin to the size of the starch granules. To settle the matter definitely more thorough investigations are required than those now undertaken. Other explanations of the differences in the grittiness of the varieties are conceivable; e.g., differences in size of the protein molecules or differences in the chemical composition of the proteins.

It may be mentioned that tests of the gluten quality were made by the Berliner-Koopmann method and showed that Eroica wheat has a higher swelling index than other South-Swedish varieties. Still, the high degree of grittiness possessed by the Eroica wheat cannot be ascribed to this fact, for a simultaneous test of Virtus wheat gave a still higher swelling index in spite of the unsatisfactory grittiness possessed by this variety.

It should be pointed out that Eroica wheat, as distinct from other winter varieties of Swedish wheat, is fairly closely allied to certain spring varieties. Bánkuter 178, the maternal grandmother of the Eroica wheat, is in all probability the result of a spontaneous cross between a Hungarian winter wheat and the spring variety Marquis. Marquis in its turn is an offspring of the Hard Red Calcutta  $\times$  Red Fife. All the Swedish spring varieties originate from Svalöf's Kolben wheat, a variety that according to Åkerman (1934) is very closely related to Red Fife. In view of the relationship between the species there is a possibility it may be the same or essentially the same hereditary factors that, independent of the protein content, are responsible



for the relatively high degree of grittiness in the majority of our spring wheat varieties as well as in Eroica wheat and in some new winter varieties now undergoing tests.

For future wheat-breeding work it is naturally of great interest that the property of grittiness has proved to be so strongly linked to the variety and is not dependent on the protein level. It will mean that this property can be freely recombined by means of systematic crossings and selection of progenies. In view of the present state of the problem of grittiness and the importance this property has for the quality of the flour, it may finally be pointed out that in future wheat-breeding work in northern Europe this property ought to receive greater attention than has hitherto been awarded it. The aim must be to ensure that new wheat varieties shall possess, in addition to other satisfactory properties, a degree of grittiness that will meet legitimate requirements.

### Summary

The milling industry requires a flour that is gritty and shows little tendency to agglomerate, and it has been generally thought that a correlation exists between grittiness and protein content.

Varieties such as Eroica have now been produced that combine high grittiness with low protein content. Investigations in which grittiness was measured by sifting tests on flours milled from both winter and spring wheats show clearly that it is variety and not the protein content that controls grittiness. Confirmatory data were obtained in trials in which protein content was varied by different levels of nitrogenous fertilizers, and no correlation between protein content and grittiness was found.

It has been observed that varieties that are gritty possess greater vitreousness of kernels in relation to protein content. Virtus and Standard with 23.8 and 19.2% vitreous and semivitreous kernels have unsatisfactory grittiness, whereas Eroica and W:s 11376 with 34.0 and 34.8% vitreousness have satisfactory grittiness. The explanation of greater vitreousness is thought to be the large amount of small starch granules in the latter two varieties, and it is possible that grittiness is correlated with this property. No correlation exists between gluten swelling index and grittiness.

Pedigrees of the varieties are discussed and it is pointed out that the hereditary factors in Swedish wheats that control grittiness may come from Red Fife, which is one of the original parents of both the winter wheat Eroica and the spring wheat Kolben.

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EXTRANEEOUS MATERIALS IN GROUND SPICES  
AND CHOCOLATE PRODUCTS<sup>1</sup>NILES H. WALKER<sup>2</sup> and GASTON DALBY<sup>3</sup>

(Received for publication April 10, 1947)

The 1945-46 Committee of the New York Section, in addition to its study of procedures for the determination of extraneous matter in flour (Walker, 1947), made a partial survey of methods applicable to other ingredients used in bakery products as well as the general condition of such samples in regard to extraneous matter. Among these, spices were found to be a serious possible source of contamination. Blumberg (1939) studied the infestation of various drugs, including many spices, with *Tribolium*. He was interested in the ability of *Tribolium* beetles to live and reproduce in the spices rather than in determining any extraneous matter that might be present. Greenish and Braithwaite (1910) published a method for the identification of insect fragments in spices which consisted of the treatment of a suspension of the spice in water with an oxidizing mixture of sulfuric and chromic acids for periods of 36-48 hours. Such a procedure would preclude the counting of rodent hairs and softer parts of insects since these would be destroyed by the reagents used. Other than the work of Blumberg, Harris (1946) in a recent bibliography of papers on sanitation lists nothing on the subject of spices and chocolate products.

The committee had in mind the following general objectives in its plan for the development of a procedure applicable to ground spices and chocolate. (Chocolate is classed with spices because of the rather general similarity in physical nature.)

<sup>1</sup> Report of the 1945-46 and the 1946-47 Committees of the New York Section on Procedures for the Examination of Food Products for Extraneous Materials.

<sup>2</sup> Chairman 1945-46 Committee. National Biscuit Company, New York, N. Y.

<sup>3</sup> Chairman 1946-47 Committee. Ward Baking Company, New York, N. Y.

1. Application of the flour procedure developed by the committee to spices without too great modification.
2. Elimination of, if possible, the necessity of removing the oily material from the spices as a preliminary step.
3. Digestion and extraction of extraneous matter under acid conditions.

A procedure for each type of food product is a source of confusion to the analyst, and the committee considered it desirable to adapt previously recommended procedures to spices with as little modification as practicable. The removal of the oils and fats from spices and chocolate products before the separation procedure is begun is time consuming; also extraneous matter may be lost at this stage unless great care is taken. Work of the Federal Security Agency (U. S. Food and Drug Administration, 1944) as well as that of the Committee indicates in general that extraneous matter is more easily and completely extracted under acid conditions than neutral or alkaline.

The recommended A.O.A.C. procedures (1945) for spices involve benzine extraction of the oil from the spice, suspension of the dry fat-free spice in water, followed by gasoline extraction of the extraneous matter using the Wildman trap technique. In the case of the *Capsicums*, the dry fat-free spice is digested in a borax solution before extraction with gasoline.

In the A.O.A.C. procedures it is necessary in many cases to work with fat-free spice because the oily spice particle is of low specific gravity and also resists being wetted by the water phase; both conditions tend to carry the spice particle over into the gasoline layer during separation of the extraneous matter. It might be said, therefore, that an oil phase is removed as a preliminary step, then an oil phase (gasoline) is added during the separation of the extraneous matter.

### Experimental

In view of these facts the committee considered the possibility of adding a solvent to the spice which would either dissolve or disperse the oil phase and permit the spice particle to be wetted by the water phase when it was added; the oil of the spice not to be removed during the procedure. Many solvents were tried, but acetone proved to have the best characteristics. In addition to its solvent properties, acetone apparently tends to loosen extraneous matter, especially rodent hairs, from the spice particles. An explanation of this action may lie in the solvent and softening effect of acetone on the resins and resinous-like material present in many spices.

Preliminary trials indicated that the acetone procedure was hopeful. Collaborative samples of Saigon cinnamon were distributed among the committee members. The objectives were to test the method in general, and specifically to find the best proportion of acetone to water and acid used. Concentrations of 40% acetone and higher had failed to show improvement in the condition of the field over lower concentrations. The boiling point of the mixture with the higher concentrations of acetone also tended to be too low for rapid digestion of materials present in many spices.

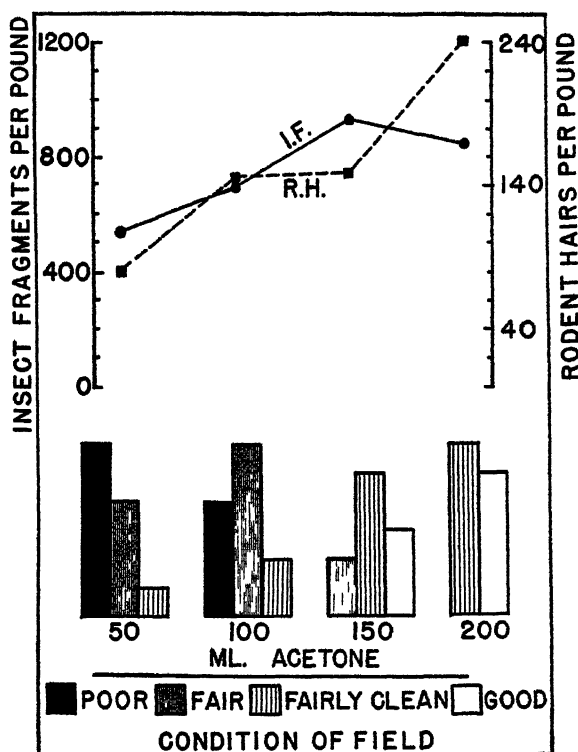


Fig. 1. Average collaborative results obtained with various quantities of acetone compared with the condition of the field as reported by the collaborators.

Ten grams of Saigon cinnamon were added to 50, 100, 150, and 200 ml of acetone in a liter Florence flask. To this were added 380 ml of water, 20 ml of concentrated hydrochloric acid, and 20 ml of light mineral oil and the mixture refluxed for about 20 minutes. The mineral oil layer was separated, washed, filtered through a lined paper, and examined in the usual manner as described by Walker (1947). The average collaborative results are shown in Figure 1. The extraneous matter counts and the condition of the fields show that 200

ml of acetone is the best quantity to use under the conditions of the test.

Six collaborators determined extraneous matter in the same sample by the A.O.A.C. procedure. The comparative results approximated to a one-pound basis are as follows:

	<i>Insect fragments per lb</i>	<i>Rodent hairs per lb.</i>
A.O.A.C. procedure	429	23
Acetone procedure (200 ml)	844	240

The tentative acetone method was then revised in accordance with the results of this study and with the suggestions of the collaborators. A rather comprehensive group of spices and chocolate products was distributed among the committee members. The objective was to test the applicability of the revised procedure to many types of products. No one collaborator was asked to test all the samples.

The committee feels that the results reported in Table I are satisfactory checks for this type of work. The objective of a test for extraneous matter is to determine whether a material is clean, lightly contaminated, or heavily contaminated. Evidence is increasing that uniform samples are not obtainable even after the most thorough mixing. The adherence of extraneous matter to spice particles could well prevent uniform distribution. Only one type of spice studied by the committee, ground sage, did not respond to the acetone procedure. The light leafy material of the sage came through into the mineral oil layer and obscured the field.

The identical sample of paprika was examined by the 1945-46 committee using the A.O.A.C. procedure and the 1946-47 committee using the acetone procedure. The average counts approximated to a one-pound basis were as follows:

	<i>Insect fragments per lb</i>	<i>Rodent hairs per lb.</i>
A.O.A.C. procedure	522	102
Acetone procedure	586	165

Once again the suggestions of the collaborators were incorporated in a final revision of the procedure.

### **Final Acetone Procedure for Determination of Extraneous Materials in Ground Spices and Chocolate Products**

Place a 10-g sample of ground spice in a dry liter Florence or similar type flask. Use a 50-g sample for cocoa and chocolate liquor. Add 200 ml of acetone and swirl flask until solvent and spice are mixed. (In the case of chocolate liquor, the sample and acetone may be re-fluxed for a few minutes at this stage so as to disintegrate the material.)

TABLE I  
RESULTS OF COLLABORATIVE STUDY OF EXTRANEOUS MATERIALS IN A  
SERIES OF SPICES AND CHOCOLATE PRODUCTS  
(Per lb. basis<sup>1</sup>)

Coll.	I.F.	R.H.	Coll.	I.F.	R.H.	Coll.	I.F.	R.H.
GROUND ALLSPICE			GROUND CARAWAY			GROUND CARDAMON		
H	1232	158	A	630	90	C	270	0
I	270	135	C	1350	90	D	1555	0
J	450	45	I	270	405	E	1271	0
GROUND CAYENNE			CHOCOLATE LIQUOR			GROUND CLOVES		
A	1170	45	D	108	0	C	270	360
C	5580	0	E	72	0	H	450	90
D	630	45	H	18	12	J	182	90
H	5130	0	J	18	0			
I	3510	90						
J	7446	90						
COCOA			GROUND CORIANDER			GROUND GINGER		
A	18	27	B	180	0	A	90	180
F	216	144	C	315	0	E	817	45
H	45	27	D	760	0	I	955	45
J	18	45						
GROUND MACE			GROUND NUTMEG			GROUND PAPRIKA		
C	4950	90	F	270	180	E	817	90
F	2475	225	G	409	57	G	704	90
I	3150	90	H	360	0	H	338	225
			J	363	0	I	630	315
						J	439	106
GROUND BLACK PEPPER			GROUND RED PEPPER			GROUND TURMERIC		
C	225	135	B	182	0	B	90	0
F	90	135	C	315	0	C	90	0
H	825	135	H	495	90	I	135	0
J	295	45	I	900	45			
			J	362	106			

<sup>1</sup> Counts approximated to a one-pound basis.

Add 380 ml of distilled or recently boiled water and 20 ml of concentrated hydrochloric acid. Finally add 20 ml of light mineral oil. Place flask under reflux condenser and boil gently for 20 minutes. Cool to about 25°C and add 50 ml of gasoline or benzine. Decant into separatory funnel. At this stage do not wash Florence flask with

water. After the heavier spice particles have settled, open clamp on separatory funnel and discard the heavy particles. Then draw off water-acetone layer, to within an inch or so of oil layer, into Florence flask and use this solution, which will contain some suspended spice particles, to wash out Florence flask. This procedure may be repeated several times. After the two layers in the separatory funnel have become well separated, draw off water-acetone layer to within an inch or so of oil layer. Thoroughly wash oil layer with water; that is, add water to separatory funnel, draw off water layer to within an inch or so of oil layer, and repeat until water layer is free from spice particles.

Filter oil layer on lined paper, wash separatory funnel and filter paper with isopropyl alcohol or ethyl alcohol, and if necessary wash paper with boiling alcohol. With many spice samples, especially those high in resins, a precipitate of amorphous material may obscure the field. In such a case, turn off suction, flood paper with 25 ml of boiling alcohol, allow to stand a few seconds, and turn on suction. Repeat if necessary. Wet paper with water, and examine under microscope in usual manner. Be sure paper is thoroughly wet during examination. For added details of washing, filtration, microscopic examination, etc., see Walker (1947).

With some spice samples, the quantity of light cellulose material carried over into the oil layer may be so great that the paper cannot be examined under the microscope. In this case wash the spice particles from the paper back into a liter Florence flask with 100 ml of acetone. Add 195 ml of water, 5 ml of concentrated hydrochloric acid, and 20 ml of light mineral oil. Reflux for 20 minutes and repeat washing procedure, filtration, etc. Filter on the same paper that was used the first time. Thirty diameters is recommended as the most convenient magnification. Use 75 diameters to check doubtful particles.

### Discussion

The samples used in this study (except the Saigon cinnamon) were chosen at random from commercial samples without any preliminary check on their degree of contamination. The results given in Table II show a serious condition as regards extraneous matter, especially in imported spices. In addition to the collaborative samples, many checks have been made by the committee members on commercial samples by the use of the acetone procedure.

Spices and chocolate are almost entirely imported products. The committee feels that the serious condition in regard to extraneous matter is not a reflection on domestic spice importers and processors. Remedial measures must come from the cooperation of the importers

TABLE II  
EXTRANEOUS MATTER FOUND IN SPICES AND CHOCOLATE  
PRODUCTS USING THE ACETONE PROCEDURE  
(Routine examination of commercial samples)

Type of material	Insect fragments per lb. <sup>1</sup>	Rodent hairs per lb. <sup>1</sup>
Allspice, Jamaica	136	0
Allspice, Jamaica	590	136
Allspice, Jamaica	0	0
Allspice, Jamaica	0	0
Allspice, Jamaica	5221	227
Allspice, Jamaica	4041	272
Allspice, Jamaica	1998	0
Allspice, Jamaica	1093	272
Allspice, Jamaica	91	136
Cinnamon, Saigon	136	227
Cinnamon, Saigon	45	0
Cinnamon, Saigon	90	45
Cinnamon, Saigon	0	0
Cinnamon, Saigon	0	0
Cinnamon, Saigon	2500	495
Cinnamon, Saigon	2000	0
Cinnamon, Saigon	3500	180
Cinnamon, Saigon	5000	1125
Cloves, Zanzibar	315	45
Cloves, Zanzibar	45	0
Cloves, Zanzibar	817	908
Cloves, Zanzibar	908	2406
Cloves, Zanzibar	999	999
Cloves, Zanzibar	0	0
Cloves, Madagascar	363	90
Cocoa	1000	27
Cocoa	500	23
Mace, West India	1226	0
Mace, Pedang	2859	363
Mace, No. 1 Siau	1589	91
Nutmeg, West India	1498	45
Paprika, Spanish	454	136
Paprika, Spanish	136	409
Paprika, Spanish	772	590
Paprika, Spanish	316	726
Paprika, Spanish	454	363
Paprika, California	91	0
Paprika, California	136	0
Red Pepper	363	45
Red Pepper	91	91
Red Pepper	454	91
Red Pepper	772	45
Red Pepper	817	0

<sup>1</sup> Counts approximated to a one-pound basis.

with various government agencies in an attempt to improve sanitary conditions in the handling, storage, and transportation of spices before they reach the United States, as well as improved domestic conditions of storage, handling, and cleaning.



### Summary

An acetone procedure for the examination of spices and chocolate products for extraneous matter has been examined collaboratively and found to be applicable. The method gives higher extraneous matter counts than previously available procedures. It is also more rapid and less laborious.

Data on the extraneous matter content of a group of spices and chocolate products are given.

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The chairmen also wish to express their gratitude to several of the committee members who helped in the preparation and distribution of samples for study.

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# THE USE OF THE AMYLOGRAPH FOR FLOUR MALT CONTROL

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(Presented at the Annual Meeting, May 1946, received for publication January 6 1947)

Discussions of the function, construction, and operation of the Brabender Amylograph have been published by Anker and Geddes (1944) and by Brown and Harrel (1944). The purpose of the present work is to consider the application of the amylograph to the control of malt flour additions to commercially milled flours and to compare the amylograph method with the methods of control now in use in the milling industry.

The maltose method of controlling these additions measures the end product of a series of enzyme reactions, since the factor determined is the reducing sugar produced by the combined actions of alpha-amylase and beta-amylase.

Within the range of enzyme concentrations customarily encountered in baking technology, the gassing power method of malt control measures the fermentable sugar produced by the same series of enzyme actions.

The amylograph method of control measures primarily the action of alpha-amylase, since the factor determined is the viscosity of the paste obtained by thermal gelatinization of a flour suspension which has been exposed to malt action. It is generally recognized that alpha-amylase is the active enzyme involved in the liquefaction of starch gels. Sandstedt (1938) showed alpha-amylase to be the prime factor in evaluation of malt in flour technology, since beta-amylase is present in excess in flour without malt supplementation.

## Experimental

The amylograph, which is essentially a recording viscosimeter, is used to measure the viscosity of a flour-water suspension that has been thermally gelled while the suspension has been and is being submitted to malt activity while undergoing constant temperature increase from room temperature to the point of maximum gel viscosity.

The procedure requires stirring 100 g of flour weighed on a 14% moisture basis, with 470 ml of McIlvaine buffer solution <sup>1</sup> adjusted to a pH of 5.35. This suspension is then treated according to the recommendations of the manufacturer for operation of the machine.

<sup>1</sup> The composition of this buffer is citric acid 0.105% and dibasic sodium phosphate 0.148%.

The amount of flour and buffer is so chosen that flour at optimum amylase activity will give a reading of between 400 and 600 amylograph units. The factor which was measured was the point of greatest consistency, as observation has indicated that this is the most important value obtained with the procedure.

The determination of the optimum concentration of malted barley or wheat flour was based on laboratory bake shop results that were obtained using the sponge-dough technique with a commercial formula. These results were then verified in three commercial bread shops in which various increments of malted barley flour were used with three bread flours of extremely low diastatic activity.

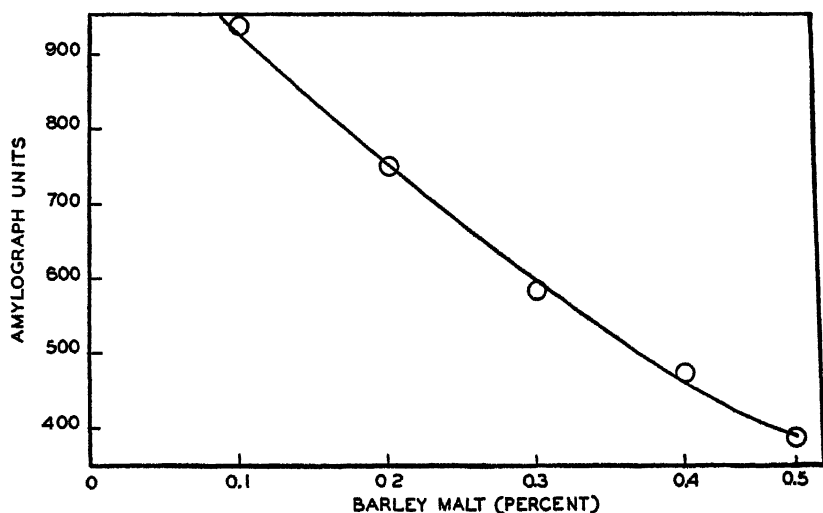


Fig 1. Relationship between amylograph value and malt concentration

The increments of malted barley flour were increased from 0.3% to 1.0% in 0.1% steps, and the qualities of the resulting dough and bread were observed. The results from the three shops indicated that the 0.4% increment of malted barley flour produced the best results, with a range of from 0.3% to 0.5% being permissible. Samples of all these flours were then submitted to amylograph analysis by the above procedure, which was designed so that the flours which produced the best results in the shop tests produced an amylograph value of 500, with flours falling within the above mentioned permissible range of amylolytic activity producing amylograph values between 400 and 600 units. This information was then applied to the control of malt flour increments for protracted periods in many commercial bakeries, with results which have in all instances verified the observations made in the original experimentation.

The relationship between amylograph value and the amount of malted barley flour added to wheat flour is presented in Figure 1. All analytical results are reported on a 14% moisture basis.

The amylograph analyses of commercial flours from several mills were compared with the maltose values or gassing power values reported by these mills.

Figure 2 shows the results of comparing the mill maltose value by the Blish-Sandstedt procedure as described in *Cereal Laboratory Methods* (4th edition, page 100), with the amylograph value determined

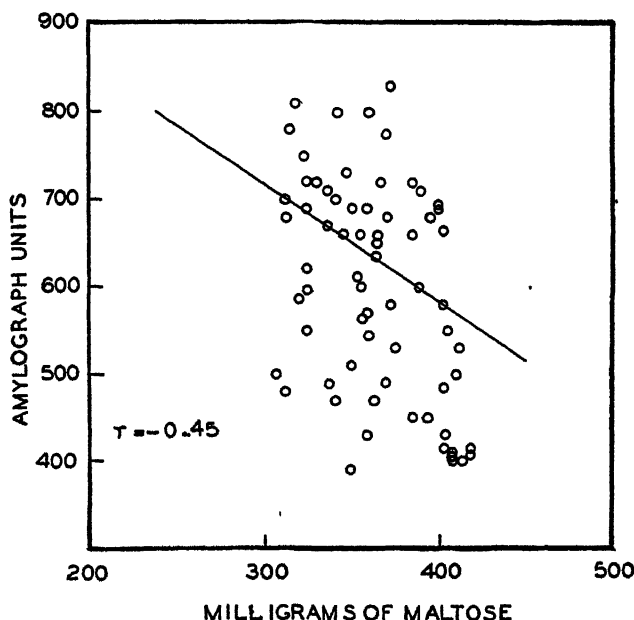


Fig. 2. Comparison of maltose values of flours from several mills with amylograph values.

in this laboratory. Sixty-nine flour samples from several mills are represented. The low significant correlation of  $-0.45$  indicates that little reliance can be placed upon the relationship between the amylograph method and the maltose method as employed by different mills.

Figure 3 is an extension of the data presented in Figure 2, and shows the maltose value plotted against the amylograph value of several samples from four mills. Although significant correlation exists in each case, it is obvious that the relationship of amylograph units to milligrams of maltose varies between mills.

Figure 4 shows the fifth hour gassing power values, determined as described in *Cereal Laboratory Methods* (4th edition, page 101), plotted against amylograph values of 44 flour samples from several mills. The

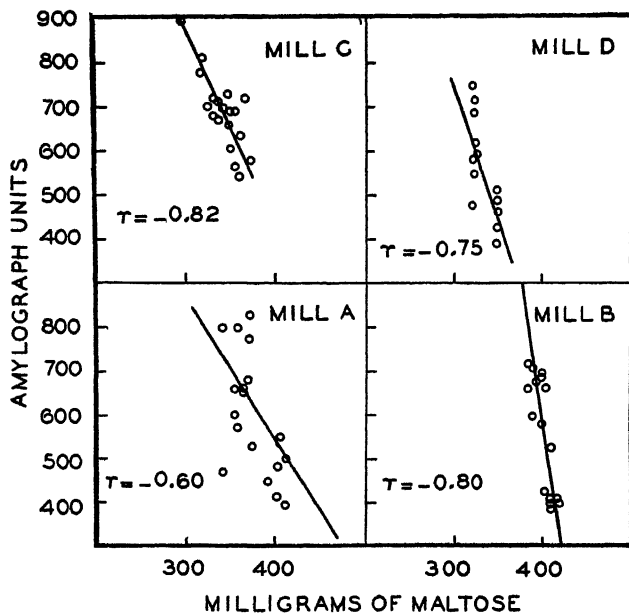


Fig 3 Comparison of maltose values of flour from individual mills with amylograph values

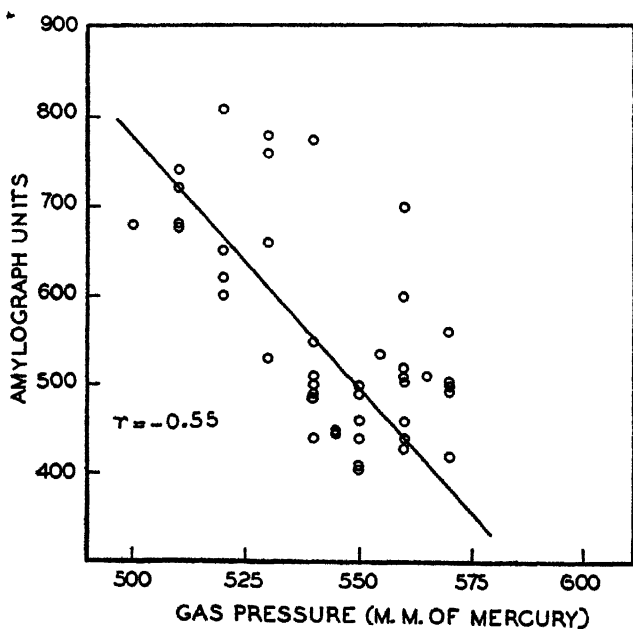


Fig 4 Comparison of fifth hour gassing power values of flours from several mills with amylograph values

low significant correlation of  $-0.55$  shown in Figure 4 indicates that a poor relationship exists between the amylograph method and the gassing power method as employed by different mills.

From these results it was obvious that, while in a given flour there may exist a correlation between the conventional methods of evaluation of amylolytic activity and the amylograph method, the nature of this relationship varies between laboratories and between flours to such an extent that in commercial practice the over-all correlation is poor. Therefore, the sensitivity of the various methods to factors known to affect the results of malt evaluation was studied. The effect of storage at various temperatures upon malt values given by the gassing power method and by the amylograph method was investigated and the effects of physical damage produced by grinding upon the values obtained by the three methods were compared.

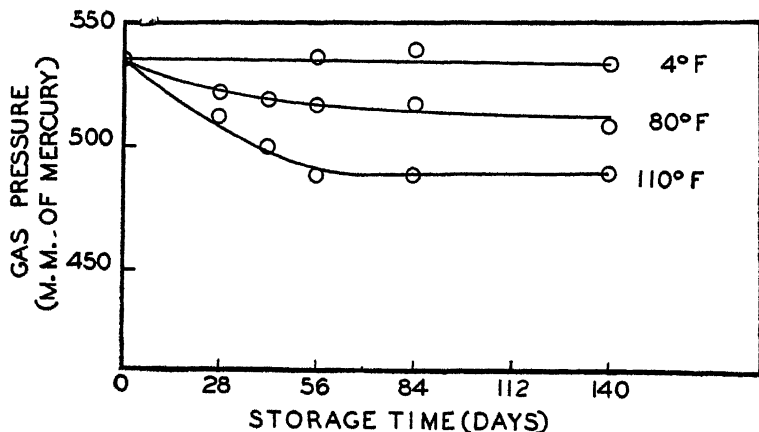


Fig. 5. Effect of storage at various temperatures upon the fifth hour gassing power of flour.

Figure 5 shows the change in gassing power of flour samples that were stored for a period of 140 days in cloth sacks at three different temperatures. Figure 6 shows the change in amylograph readings on the same storage samples.

Table I shows the results of overgrinding upon the amylolytic value of the flour as determined by three methods. The flour was ground with steel balls in a porcelain ball mill.

The amylograph value was affected to some extent by 30 hours of continuous grinding, but the indicated amylolytic value of the flour did not fall outside the optimum baking range.

Seven hours of grinding produced a gassing power sufficient to indicate considerable overtreatment of the flour. Fifteen hours of

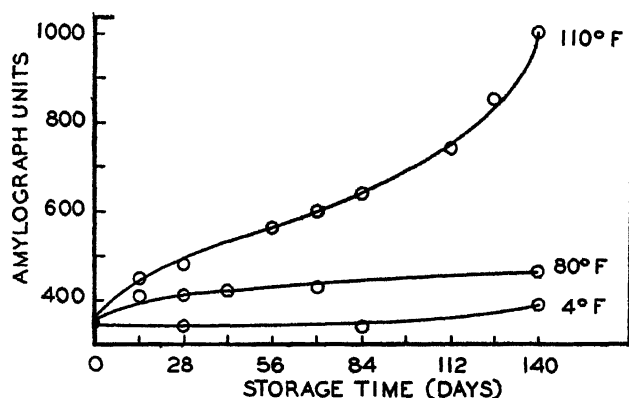


Fig. 6. Effect of storage at various temperatures upon the amylograph value of flour.

grinding increased the maltose value beyond the limits of commercial flours.

Since damaged granules are always produced in commercial milling, these results may give some indication of the reason for the lack of correlation between the amylograph procedure and the conventional methods of malt control used by mills. A slight difference between mills in extent of milling damage can produce an effect upon maltose and gassing power values which will not be shown proportionately by the amylograph method.

TABLE I  
EFFECT OF GRINDING UPON THE APPARENT AMYLOLYTIC ACTIVITY  
OF WHEAT FLOUR AS MEASURED BY VARIOUS METHODS

Grinding period (hours)	Amylograph units	Gas pressure (mm of mercury)	Milligrams of maltose
0	595	553	304
7	560	610	398
15	485	639	436
30	440	642	502
Optimum range	400-600	540-575	370-400

### Discussion

The complete role of alpha-amylase in the baking of bread has been studied by a few workers. Kneen and Sandstedt (1946) and Geddes (1946) have discussed the possible functions other than sugar production which this enzyme may have in bread manufacture. Certainly it would seem that if the production of sugar were the primary or most important work of amylase, then a baker should be able to maintain gas production in the dough and produce high quality bread with a total disregard of the amylase activity simply by adjusting the quantity

of sugar in the formula. Such an adjustment has not been found to substitute for amylase activity in producing maximum loaf volume and optimum texture.

As every baker has discovered, gas retention is as important as gas production, and there has been no evidence found in this laboratory to show that gas retention of fermenting dough is affected markedly by amylase. The writers have always associated gas retention with proper conditioning of the flour gluten.

There is strong evidence that places the emphasis on the final effect of the amylases in the oven as being the most important function of malt. Not all of the activity of the amylases takes place in the oven, but the fermentation of the dough piece before entering the oven allows amylolytic activity to condition properly the starch so that when a gel is formed in the oven it will be of the optimum viscosity with the correct level of alpha-amylase activity.

It would seem quite possible that a large portion of the total effect of the amylase on the starch granules actually takes place in the oven during the first stage of baking. The work of Anker and Geddes (1944) indicated that alpha-amylase activity lowers the gelatinization temperature of wheat starch suspensions, as well as decreasing the maximum viscosity reached during gelatinization. If it is assumed that an important function of alpha-amylase is to prepare the starch so that it will gelatinize at the right temperature and to the correct viscosity, then it would be expected that flour deficient in alpha-amylase activity would produce a gel of high viscosity in the oven and that as the exterior of the loaf, which was first exposed to the heat, gelled, the expansion of the loaf would be reduced and the resultant loaf would be small in volume and characterized by a coarse texture.

On the other hand, if the flour is overtreated, then the low viscosity of the starch gel will not establish securely the structure of the loaf and here again there will be some diminution of volume, coalescing of the cellular structure of the crumb, and a dark crumb color.

If the flour has been treated to the optimum range, then the starch viscosity will be low enough to allow for the expansion of the loaf and yet will be high enough so that the established cell structure is of fine and desirable grain.

Although to some extent it is oversimplifying the problem, the writers assume that conditioning of gluten is extremely important for the formation of a finely subdivided dough structure; that, when the temperature reaches 75° to 85°C during baking, the gelling starch assumes the burden which to this point has been carried by the gluten; and that the importance of the gluten as a structural unit is greatly reduced as the starch gel takes over.



Several experiments would appear to justify the conclusion given above. Obermeyer (1940) reconstituted synthetic dough in which gluten was mixed with small particles of glass, round in shape and fractionated to the same size range as wheat starch granules. A synthetic dough was made with yeast, salt, and a small amount of sugar. Except for the coloration of the dough it was normal in appearance during the panary proof and during the very first stages of baking. That is, there was a considerable amount of oven spring; however, after the initial increase in oven spring, the entire dough collapsed to the bottom of the pan as a rubbery mass.

Selman (1942) found that, when doughs of very high absorption (100 to 125%) were baked by means of electrical resistance, they all had the typical appearances of normal doughs until a temperature of 76°C was reached, at which point they abruptly became converted into bread although less than 1% of the total water had been evaporated at this stage.

Continued experimentation with doughs of normal absorption ranges indicated that the same phenomenon occurred. However, the change from "dough" characteristics to "bread" characteristics occurred over a much wider range of temperature and was not nearly so sharp as in the doughs containing excess amounts of water.

All of these results indicated that at this stage of bread manufacture the size and texture of the finished loaf are dependent upon the gelatinization characteristics of the starch. Control of this property is, in the authors' opinion, the most important function of alpha-amylase in the bread baking process.

### Summary

A method for the use of the amylograph for flour malt control is presented. The relation between this method and the maltose method was found to be generally poor. The same was found to be true of the relation between the amylograph method and the gassing power method.

The amylograph method was shown to be more sensitive to changes in malt activity and less sensitive to milling damage than either of the other methods.

The amylograph method is better suited to measure directly the primary effects of malt during the baking process than the maltose method and the gassing power method.

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## COMMUNICATION TO THE EDITOR

### A Corrected Unit of Alpha-Amylase Activity

SIR:

Shortly after the publication of our paper, "Modification of the Kneen and Sandstedt Methods for the Determination of Alpha- and Beta-Amylases in Barley Malt," *Cereal Chemistry* 21: 533-539 (1944), we realized that an error had been included in the method of calculation of alpha-amylase activity.

In contrast to the unit of alpha-amylase activity originally proposed by Sandstedt, Kneen, and Blish (1939), the activity unit proposed in our work was based on an experimentally determined relationship between the dextrinizing and saccharifying properties of alpha-amylase. This relationship is given in Figure 1, page 536, of the above paper. The function, found to be linear over the range studied, did not pass through the origin; the method of calculation was based on the assumption that it did.

All attempts to redetermine the function resulted in curves of the same general type as that illustrated. The error over the entire range of the curve amounts to 15 maltose equivalents (4° Lintner), which is well within the limits of accuracy of the methods employed for the determination of saccharifying activity, but makes the values unsuitable for use as a measure of alpha-amylase activity.

The use of the relationship illustrated in Figure 1 and the values presented in Table I to correct the diastatic activity value for alpha-amylase saccharification, thereby obtaining values for beta-amylase, will not result in errors greater than those of the diastatic power method.

Therefore, we recommend the abandonment of the unit of alpha-amylase we originally proposed. We recommend further that a unit similar to that of Sandstedt, Kneen, and Blish (1939) be adopted, to wit: The alpha-amylase units are the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 20°C, this unit to be called "20° dextrinizing unit."

Inasmuch as the only essential difference between the Nebraska methods and our modification is the temperature of extraction of the malt and of the dextrinization, it seemed desirable to establish a conversion factor between 20° and 30°C dextrinizing times. It was found that the conversion factor obtained with 30 barley malts<sup>1</sup> was not constant, often varying from the mean by 20%. Therefore, at present, we cannot recommend the use of a conversion factor between values obtained by the 20° and 30°C methods.

The calculations of the activities are the same as reported in our paper, except that alpha-amylase is calculated as indicated below:

$$\text{D.P. (maltose equivalent, dry basis)} = \frac{(\text{blank-titration}) \times 144 \times 100}{(100 - \% \text{ moisture})}$$

$$\begin{aligned} \text{Alpha-amylase activity (20° dextrinizing unit dry basis)} = \\ \frac{0.4 \times 60 \times 100}{\text{malt equiv. (g)} \times \text{dext. time} \times (100 - \% \text{ moisture})} \end{aligned}$$

Beta-amylase:

Correction for alpha-amylase saccharification (A)

$$\begin{aligned} A &= \frac{K \times 100}{(100 - \% \text{ moisture}) \times \text{ml diastatic extract used in dextrinization} \\ &\quad \text{and beta-amylase activity (20°C) maltose equivalents,} \\ &\quad \text{dry} = \text{D.P.} - A. \end{aligned}$$

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Madison, Wisconsin

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<sup>1</sup> The results of this study will be submitted for publication shortly.

## BOOK REVIEWS

**Air Conditioning in the Baking Industry.** By J. R. Seville. 152 pp. MacLaren & Sons, Ltd., London. 1946. Price 25 shillings in Great Britain.

This book deals with air conditioning from the baker's point of view. It attempts to give the "why" as well as the "how" as regards temperature and humidity controls. It contains 38 figures which are largely excellent photographic reproductions of equipment in use for recording and controlling the factors under discussion and including also bakery machinery used in the various stages of dough processing.

Section one of the book is introductory and general. It contains the following chapters: (1) Introduction, (2) The Basic Principles of Air Conditioning, (3) The Measurement of Humidity, (4) Obtaining the Required Humidity.

Section two of the book deals with Air Conditioning Applied to Breadmaking Processes. Each process or department is considered in a separate chapter and in the following order: (5) Flour Storage, (6) Doughroom, (7) First Proof, (8) Final Proof, (9) Oven, (10) Cooler, (11) Bread Storage. A unique part of this section of the book is the division of each chapter into two parts. The first part includes a considerable discussion of changes which occur during this particular part of the breadmaking process and the importance of temperature and humidity control in obtaining best results. The second part of each chapter describes suitable air conditioning methods and equipment for use in this operation. Chapter 12 contains notes on the Care of Air Conditioning Equipment. A glossary of terms most frequently used in air conditioning engineering is included. Tables are also given for temperature conversion, for finding relative humidities from wet and dry bulb readings, for showing relationships between velocity pressure and air velocity, and for pressure effects upon temperature, heat value, and volume of steam.

The author shows considerable familiarity with American methods and equipment. Much equipment from manufacturers in America and Britain is in use in both countries so the overseas authorship does not limit its usefulness on this side of the Atlantic. Descriptions of processes and equipment are clearly given in relatively simple language. Many cereal chemists, as well as bakers, might find this book to be a source of useful information. There may not be agreement with the author's opinions on some of the changes occurring in breadmaking. His views are rather positively stated, but this gives the presentation a certain simplicity.

OSCAR SKOVHOLT

Quality Bakers of America Cooperative, Inc.,  
New York, N. Y.

**Handbook of Analytical Methods for Soybeans and Soybean Products.** National Soybean Processors Association, 3818 Board of Trade Bldg., Chicago, Illinois. 1946. 40 pp. Price \$1.00.

This handbook is an example of a commendable practice that is gaining favor with trade associations. To facilitate the handling of raw and processed materials and to protect buyers and sellers in contracts for future delivery, such trade associations have set up specifications and trading rules, and have chosen official referees. To complete such a program it is necessary to stipulate precise directions for carrying out necessary analytical procedures; this handbook represents such a compilation for use by soybean processors.

The methods given are conventional procedures that fall within the limitations established by such organizations as the Association of Official Agricultural Chemists and the American Oil Chemists Society, but for the most part are given more specifically than is the case with methods published by the latter organizations. The A.O.A.C. and A.O.C.S. must necessarily write their official procedures in such a manner that some latitude is allowed for the judgment of the analyst in order that the general procedures may be satisfactory for use with a variety of products.

The one exception noted to the avowed editorial policy of eliminating alternative steps or procedures is in the determination of crude protein. Here the Kjeldahl-Gunning-Arnold method as given by the A.O.A.C. has been specified. It appears to the reviewer that for trading purposes the National Soybean Processors Association might well adopt a version of this method which would meet A.O.A.C.

requirements in all respects but which would be more specific in regard to such items as size of sample, choice of catalyst, and quantities of reagents to be used.

The Technical Committee of the N.S.P.A., which was responsible for the preparation of this handbook, has included several methods for the analysis of soy flour and lecithin which are not to be found in "official" compilations of methods. These procedures have proved their value in various plant laboratories, and should be welcomed particularly by those who have occasion to analyze soy products infrequently.

The style employed for presentation of methods is clear, and the booklet appears to be remarkably free from typographical errors.

F. C. HILDEBRAND  
General Mills Inc.  
Minneapolis, Minnesota

**Concise Chemical and Technical Dictionary.** Edited by H. Bennet. xxxix + 1055 pp. Chemical Publishing Co., Inc., Brooklyn, New York. 1947. Price \$10.00.

This book aims at providing concise definitions of about 50,000 terms employed in every technical and scientific field. It includes thousands of trade-name or proprietary products together with a description of their chemical and physical properties and chemical composition. Several tables are included as well as an addenda listing the latest trade names and definitions of additional technical terms.

The system of nomenclature used for organic compounds is based on that recommended by the International Union of Chemistry.

The book is well printed but it is felt that greater consistency could have been observed in the cross references. Further, after using the dictionary for several weeks it is felt that it lacks many useful technical and scientific terms, especially with regard to cereal chemistry and the milling and baking industries.

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## COMPARATIVE BIOLOGICAL AVAILABILITIES OF VARIOUS FORMS OF IRON IN ENRICHED BREAD

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The federal and state programs of flour and bread enrichment have made the biological availability of iron a subject of considerable importance to the milling, baking, and allied industries, as well as to the general public. Despite the importance of the problem, only a few studies have been published on the comparative biological values of the various forms of iron commonly used in bread enrichment. In order to be satisfactory for addition to dough mixes or to flour, an iron preparation must have no deleterious effect upon the quality of the bread or flour. Moreover, the iron should also be satisfactorily assimilable by the body.

Since the iron occurring naturally in wheat is considered to be associated largely with phytic acid, a phytate salt was one of the first types of iron suggested for bread enrichment. The iron of ferric phytate was reported by Andrews, Evans, and Huber (1941) to be utilized as well as saccharated iron oxide for hemoglobin formation when tested in iron-deficient anemic rats. Nakamura and Mitchell (1943) found that the utilization of ferric phytate in rats was comparatively poor, only about half that of the highly available ferric chloride. In human subjects, McCance, Edgcombe, and Widdowson (1943) observed that the presence in bread of sodium phytate, and to a less extent disodium phosphate, decreased the absorption of iron. Moore, Minnich, and Dubach (1943) found that ferric phytate, administered alone or in bread, was much less readily absorbed than ferrous sulfate both in normal human subjects and in patients with hypochromic microcytic anemia. Because of the relatively poor absorbability, these latter investigators concluded that ferric phytate was not a satisfactory form of iron for the enrichment of food.

Conflicting results have been reported in rat experiments on sodium iron pyrophosphate. Nakamura and Mitchell (1943) concluded that

it was as well utilized as reduced iron or ferric chloride. On the other hand, Street (1943) found sodium iron pyrophosphate to be a comparatively poor source of iron, less than 50% as effective as the highly available ferrous sulfate or ferric sulfate. Freeman and Burrill (1945) also reported sodium iron pyrophosphate to be poorly utilized, i.e., less than 50% as effective as ferric chloride. They found that sodium ferric orthophosphate, ferric orthophosphate, and reduced iron gave slightly lower values for availability than ferric chloride, but these differences were not significant.

The literature on this subject is not only incomplete, but also contains some important discrepancies. In view of this, the present investigation was undertaken to secure additional information on the comparative biological availabilities of various forms of iron in enriched bread, as determined by hemoglobin regeneration in rats made anemic from iron deficiency. The iron preparations were tested in the form of enriched breads baked with iron sources of current commercial interest, namely, ferrous sulfate ( $\text{FeSO}_4$ ), ferric orthophosphate ( $\text{FePO}_4$ ), reduced iron, and sodium iron (ferric) pyrophosphate ( $\text{Fe}_4(\text{P}_2\text{O}_7)_3 \cdot 2\text{Na}_4\text{P}_2\text{O}_7 \cdot 6\text{H}_2\text{O}$ ).

## Experimental Methods and Results

### EXPERIMENT 1

*Diets.* The basal diet used in this experiment was patterned generally after that of Street (1943) and had the following percentage composition: dried bread 77, casein (low-iron) 12, salt mixture (low-iron) 3, and peanut oil 8. Supplements incorporated were, in mg per 100 g of diet: thiamine hydrochloride 1, riboflavin 2, pyridoxine hydrochloride 1, calcium pantothenate 4, niacinamide (nicotinamide) 2, choline chloride 100, inositol 100, copper (as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) 3, and manganese (as  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ) 1.5. Each rat received by stomach tube a weekly fat-soluble vitamin supplement equivalent to 2000 U.S.P. units of vitamin A, 400 U.S.P. units of calciferol (vitamin  $\text{D}_2$ ), and 10 mg of  $\alpha$ -tocopherol.

Since bread itself does not provide adequate protein, casein was included in the diet. The low-iron casein, which was prepared in the laboratory from skimmed milk, had an iron content of approximately  $20\mu\text{g}$  per g. The low-iron salt mixture was prepared by modifying U.S.P. XI Salt Mixture No. 2. Ferric citrate was omitted. Since ample sodium chloride was supplied by the bread, sodium chloride also was omitted from the salt mixture and the amount of salt mixture was reduced from the usual 4% of the diet to 3%. Furthermore, the sodium biphosphate was replaced by potassium biphosphate.

All of the test breads were baked from the same lot of flour with special enrichment mixes that supplied the usual amounts of thiamine, riboflavin, and niacin, but varied with respect to iron. One lot of bread contained no added iron; the others were enriched by approximately 11 mg per lb (24.2  $\mu$ g per g), dry weight, with the various forms of iron shown in Table I. This level of iron is within the range used in

TABLE I  
IRON CONTENTS OF DIETS

Expt.	Group	Diet	No. rats	Iron content of bread	Iron content of diet
				$\mu$ g/g	$\mu$ g/g
1	1	Negative control bread (no added iron)	16	18.1	17.5
	2	Ferrous sulfate	15	41.9	35.8
	3	Ferric orthophosphate	15	40.7	34.9
	4	Reduced iron	15	38.0	32.8
	5	Sodium iron pyrophosphate	16	41.6	35.6
	6	Positive control (added iron 200 $\mu$ g/g)	11	—	217.5
	7	Milk negative control	11	—	—
2	1	Negative control bread (no added iron)	17	11.8	14.2
	2	Ferrous sulfate	19	33.3	31.3
	3	Ferric orthophosphate	18	36.2 <sup>1</sup>	31.1
	4	Reduced iron	19	32.9	31.0
	5	Sodium iron pyrophosphate	19	33.3	31.2
	6	Positive control (added iron 200 $\mu$ g/g)	9	—	214.2

<sup>1</sup> Adjusted to 33.0 by slight dilution with negative control bread.

commercial enrichment. The iron samples themselves were analyzed and incorporated into the mix according to the iron content found. The reduced iron (U.S.P.) and ferric orthophosphate were from batches actually used for commercial enrichment; the ferrous sulfate was U.S.P. grade, and the sodium iron pyrophosphate was of a quality suitable for enrichment. After being air-dried at 37°C to a moisture content of approximately 4%, the breads were ground for use in the diets. Diet 6 was prepared by addition of ferrous sulfate to the negative control Diet 1 at a level of 200  $\mu$ g of iron per g of diet. This provided a positive control to determine the maximum rate of hemoglobin regeneration permitted by the basal diet in the presence of a known optimal amount of available iron.

The actual iron contents of the various breads and diets are given in Table I. The iron analyses were made by a thiocyanate procedure (Eckert and Auerbach, 1944). The bread component contributed about 91% of the iron in the diet, 51% being from the enrichment mix and 40% from the iron present in the unenriched bread.

*Animal Experimentation.* Albino rats of the Sherman strain were prepared for iron-deficiency studies by special feeding precautions



generally similar to those described by Elvehjem and Kemmerer (1931). When weaned at about 24 days of age, the young rats were removed to new, individual, galvanized cages in which there was no exposed iron or rust. Anemia was induced in the animals by the feeding of certified cow's milk to which had been added cupric sulfate and manganous sulfate equivalent to 1.8 mg of copper and 1.4 mg of manganese per liter of milk. A few animals died during the depletion period. After 31 days of iron depletion, hemoglobin determinations were made on tail blood by the alkaline hematin method, as adapted for the Klett-Summerson colorimeter. Except for 11 somewhat resistant animals with hemoglobin values of 5–10 g per 100 ml, the rats were found to be sufficiently anemic for test purposes, i.e., had hemoglobin values of 2–5 g per 100 ml, with an average of about 2.9 g.

As shown in Table I, the animals were divided into seven groups of 11–16 rats each, with similar weight and sex distributions. The 11 less anemic rats formed Group 7, which was continued on the anemia-producing milk diet as another form of negative control. The experimental diets were then fed for four weeks, hemoglobin determinations and weighings being made at the end of each week.

**Results.** The hemoglobin regeneration curves are shown in Figure 1. Inasmuch as some of the animals in the highest test groups reached

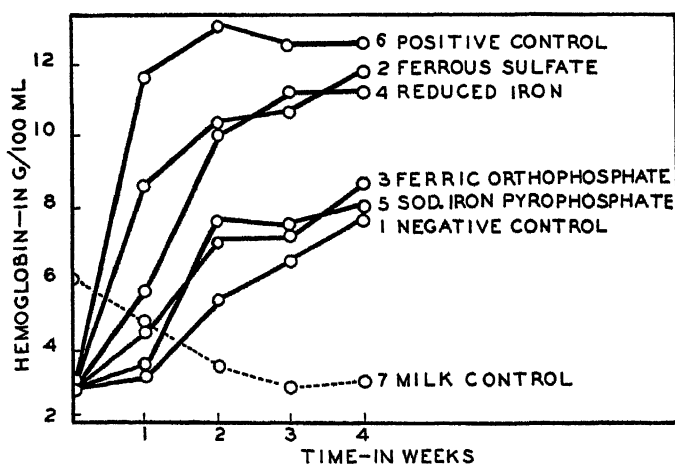


Fig. 1. (Experiment 1) Hemoglobin regeneration curves in anemic rats fed various iron source materials in form of enriched bread.

the normal range of hemoglobin values between the first and second weeks, the interpolated value for  $1\frac{1}{2}$  weeks appeared to be the most sensitive point for comparison. When some later point on the curve is used, the quantitative superiority of the more highly available forms

becomes somewhat masked because the animals that had already reached normal hemoglobin values by the second week were capable of little or no further gain. On the other hand, when the results at one week are used, the inferiority of the poorly available forms may be exaggerated due to an initial physiological lag in response, as evidenced by a sharp increase in the slope of the curve between the first and second weeks. The general trend of the results at  $1\frac{1}{2}$  weeks is confirmed by the curves in Figure 1 for other points during the four-week test.

The initial mean hemoglobin values, as listed in Table II, indicated that the four test groups were at approximately the same degree of anemia at the start of the experiment. Mean hemoglobin gains at  $1\frac{1}{2}$  weeks were calculated. However, since there were minor differences in the iron contents of the four test breads (cf. Table I), these mean values were corrected slightly on the basis of the iron content of Diet 2 (ferrous sulfate), which was highest in iron. The corrected means and standard errors are given in Table II. From the hemoglobin regeneration curves and the values for mean hemoglobin gains, it may be seen that the iron sources compared as follows, in order of decreasing availability: ferrous sulfate > reduced iron > ferric orthophosphate > sodium iron pyrophosphate.

The statistical significance of these differences was determined by calculation of the standard error of the difference between the mean hemoglobin gains at  $1\frac{1}{2}$  weeks, comparisons being made for all groups. A relatively high criterion of significance was adopted by basing conclusions only on P values of 0.01 or less (i.e., probability of difference being fortuitous equals 1 in 100, or less). As may be seen in Table II, the superiority of ferrous sulfate over ferric orthophosphate and sodium iron pyrophosphate was highly significant ( $P = <0.001$ ), but its superiority over reduced iron was not quite significant ( $P = 0.02$ ). Reduced iron also was significantly superior to ferric orthophosphate and to sodium iron pyrophosphate ( $P = <0.001$ ). Ferric orthophosphate was not significantly superior to sodium iron pyrophosphate ( $P = 0.62$ ).

The values for total hemoglobin gains at four weeks were also analyzed, although this is not a sensitive point for comparison. Ferrous sulfate was not significantly different from reduced iron ( $P = 0.86$ ). However, even at this time both ferrous sulfate and reduced iron were still significantly superior to ferric orthophosphate and sodium iron pyrophosphate ( $P = <0.001$ ), as shown in Table II. Ferric orthophosphate was not significantly superior to sodium iron pyrophosphate ( $P = 0.46$ ).

The foregoing analysis established the order of superiority among the test forms of iron used in these experiments. For convenience,

TABLE II  
HEMOGLOBIN REGENERATION AND GROWTH OF RATS ON DIFFERENT FORMS OF IRON

Expt.	Group	Diet	No. rats	Initial wt. (avg.) g	Wt. gain (avg.)		Hemo- globin ini- tial mean g/100 ml	Hemoglobin gain at 1½ weeks		Hemoglobin gain at 4 weeks		Availa- bility %
					1½ wks.	4 wks.		Mean ± S.E.	P <sup>1</sup>	Mean ± S.E.	P <sup>1</sup>	
1	1	Negative control bread	16	75	g	g	g/100 ml	1.61 ± 0.33	<0.001	4.89 ± 0.40	<0.001	—
	2	Ferrous sulfate	15	70	25	78	2.89	6.55 ± 0.31	—	8.98 ± 0.40	—	100
	3	Ferric orthophosphate	15	73	32	89	2.85	3.04 ± 0.44	<0.001	5.82 ± 0.66	<0.001	29
	4	Reduced iron	15	75	25	82	2.89	5.38 ± 0.36	0.02	9.09 ± 0.49	0.86	76
	5	Sodium iron pyrophos- phate	16	72	33	91	2.88	2.77 ± 0.31	<0.001	5.24 ± 0.40	<0.001	23
	6	Positive control	11	61	25	77	3.10	9.24 ± 0.59	—	9.40 ± 0.47	—	—
2	1	Negative control bread	17	72	30	81	4.31	0.72 ± 0.26	<0.001	2.90 ± 0.36	<0.001	—
	2	Ferrous sulfate	19	72	45	104	4.46	5.13 ± 0.21	—	10.20 ± 0.27	—	100
	3	Ferric orthophosphate	18	73	37	97	4.14	2.78 ± 0.31	<0.001	6.68 ± 0.33	<0.001	47
	4	Reduced iron	19	68	39	102	4.36	4.70 ± 0.30	0.25	9.87 ± 0.32	0.43	90
	5	Sodium iron pyrophos- phate	19	73	38	98	4.35	1.54 ± 0.28	<0.001	5.84 ± 0.29	<0.001	19
	6	Positive control	9	66	42	112	2.88	9.64 ± 0.27	—	12.90 ± 0.28	—	—

<sup>1</sup> P = probability of fortuitous difference (from ferrous sulfate).

rather than for statistical accuracy, it was desirable to express the comparative availabilities quantitatively. This was done on the basis of mean hemoglobin gains at  $1\frac{1}{2}$  weeks. The effect due to the added iron was computed by subtracting from each value the amount of gain that resulted on the negative control diet alone. In Table II the availabilities of the types of iron are rated with reference to ferrous sulfate as 100%.

There were only slight differences in the weight gains of the four test groups, and these minor differences did not correlate with the large variations in hemoglobin regeneration (cf. Table II). An extra copper supplement failed to improve the hemoglobin regeneration in three additional rats fed the sodium iron pyrophosphate diet, thereby indicating that the poor response could not be attributed to an inadequacy of copper in the diet.

## EXPERIMENT 2

*Diets.* For confirmation of the results secured in the first comparative test, a second experiment was conducted under closely similar conditions. New enrichment mixes were prepared. The second series of breads was enriched by approximately 10 mg of iron per lb (22  $\mu$ g per g), dry weight, with the forms of iron shown in Table I. This level of iron was by intention slightly below the 11 mg per lb used in the first experiment, but still within the legal enrichment range. The samples were coded both in this laboratory and at the bakery, so that the identities of the breads were not known until after the conclusion of the experiment.

The basal diet differed from that of the previous experiment only in that the bread was increased from 77% to 82% at the expense of the peanut oil. Since the level of iron enrichment was lower in this series, it was considered safe to use the test component, bread, at its maximum proportion in the diet without introducing an amount of ferrous sulfate above the minimum for optimal response. The iron contents of the breads, as found by chemical analysis, are given in Table I. All of the iron-enriched samples were approximately 33  $\mu$ g per g, dry weight, except Bread No. 3, which gave the slightly higher value of 36.2  $\mu$ g per g. Therefore, Bread No. 3 was diluted slightly by addition of the negative control Bread No. 1 to give an iron content of 33  $\mu$ g per g also. The bread component contributed about 87% of the dietary iron, 56% being from the enrichment mix, and 31% representing the iron present in the bread even when iron is omitted from the enrichment mix (e.g., Bread No. 1).

*Animal Experimentation.* Sherman strain albino rats were made anemic as previously described, although the anemia was not allowed

to become so severe as in Experiment 1. After 26 days of iron depletion, almost all of the rats had hemoglobin values of 2.5–5.0 g per 100 ml (average about 4.3 g), so that they were sufficiently anemic for test purposes. The animals were divided into six groups of 9–19 rats each, as shown in Table I. The experimental diets were then fed for four weeks, hemoglobin determinations and weighings being made at the end of each week. Group 1, the negative control, was continued for two additional weeks to determine how much time would be required to reach a hemoglobin value of 10 g per 100 ml, i.e., close to the beginning of the normal range.

**Results.** The general nature of the results is shown by the curves of hemoglobin regeneration in Figure 2. Mean hemoglobin gains at

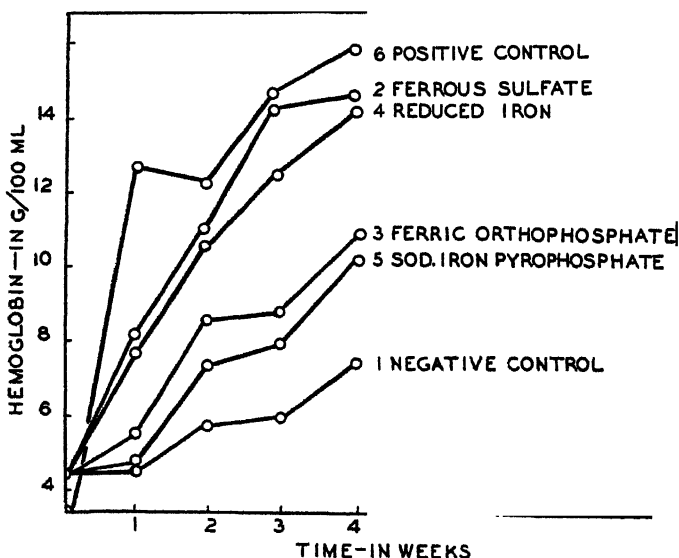


Fig. 2. (Experiment 2) Hemoglobin regeneration curves in anemic rats fed various iron source materials in form of enriched bread.

1½ weeks are given in Table II. The general trend of the results at this point is confirmed by the curves in Figure 2 for other points during the four-week test. In confirmation of the results of the first experiment, the iron sources again compared as follows, in order of decreasing availability: ferrous sulfate > reduced iron > ferric orthophosphate > sodium iron pyrophosphate.

The mean hemoglobin gains at 1½ weeks were analyzed statistically, with the previously mentioned P value of 0.01 or less as the criterion of significance. As may be seen in Table II, the superiority of ferrous sulfate over ferric orthophosphate and sodium iron pyrophos-

phate was again highly significant ( $P = <0.001$ ), but its superiority over reduced iron was not significant ( $P = 0.25$ ). Reduced iron was again significantly superior to ferric orthophosphate and sodium iron pyrophosphate ( $P = <0.001$ ). In this second experiment the superiority of ferric orthophosphate over sodium iron pyrophosphate ( $P = 0.005$ ) was slightly above the criterion of significance (apparently because the value at two weeks was unduly high with respect to the rest of the curve, as may be seen in Figure 2).

The values for total hemoglobin gains at four weeks were also analyzed, although, as mentioned previously, this is not a sensitive point for comparison. Ferrous sulfate was not significantly different from reduced iron ( $P = 0.46$ ). However, at this point also the superiority of ferrous sulfate and of reduced iron over ferric orthophosphate and sodium iron pyrophosphate was highly significant ( $P = <0.001$ ), as shown in Table II. At the four-week point, the superiority of ferric orthophosphate over sodium iron pyrophosphate was not significant ( $P = 0.066$ ).

For an approximate quantitative comparison, the percentage availabilities were again calculated on the basis of the hemoglobin gains at  $1\frac{1}{2}$  weeks (cf. Table II). These figures were in general agreement with those of the first experiment. The various forms of iron were also compared on the basis of the number of days required to reach an average hemoglobin value of 10 g per 100 ml, which is close to the normal range (Table III). They were then rated for percentage

TABLE III  
EXPERIMENT II. AVAILABILITIES OF FORMS OF IRON, BASED UPON DAYS  
REQUIRED TO REACH HEMOGLOBIN VALUE OF 10 G PER 100 ML

Group	Diet	Days	Availability (%)
2	Ferrous sulfate	11.9	100
4	Reduced iron	12.7	96
3	Ferric orthophosphate	24.8	46
5	Sodium iron pyrophosphate	27.7	34
1	Negative control bread	35.9	0

availability in comparison with ferrous sulfate. The results are in approximate agreement with the percentage availabilities found by the previous method (cf. Table II, Experiment 2).

In striking contrast to the wide variations in hemoglobin regeneration, the weight gains of the four test groups differed little. The differences in food consumption and iron intake were very small and could not account for the large differences in hemoglobin regeneration (cf. Table IV). As a matter of fact, even these small differences were

TABLE IV  
EXPERIMENT II. HEMOGLOBIN GAINS, FOOD CONSUMPTIONS,  
AND IRON INTAKES OF RATS

Group	Diet	For 1½ weeks (avg.)			For 4 weeks (avg.)		
		Hemo- globin gain	Food consump- tion	Iron intake	Hemo- globin gain	Food consump- tion	Iron intake
		g/100 ml	g/day	µg/day	g/100 ml	g/day	µg/day
1	Negative control bread	0.72	9.4	134	2.90	9.2	131
2	Ferrous sulfate	5.13	11.6	363	10.20	12.4	388
3	Ferric orthophosphate	2.78	10.8	336	6.68	12.2	379
4	Reduced iron	4.70	11.3	350	9.87	12.5	387
5	Sodium iron pyrophosphate	1.54	10.9	341	5.84	12.2	382
6	Positive control	9.64	11.8	2528	12.90	12.9	2761

somewhat compensated for by the corresponding slight differences in growth. Furthermore, two rats each on the reduced iron diet and on the sodium iron pyrophosphate diet were paired-fed to maintain individual food consumption the same. The reduced iron group again showed the expected superiority.

For an over-all approximate comparison, the percentage availabilities in both experiments, as given in Table II, were averaged. The following results were obtained: ferrous sulfate 100, reduced iron 83, ferric orthophosphate 38, and sodium iron pyrophosphate 21.

### Discussion

The experiments described in this report were designed to compare the iron preparations in a manner simulating as closely as possible the practical conditions of use in the enrichment program, i.e., actually baked in bread at the prescribed legal levels. The iron intake was at a normal or physiological level for rats, rather than in a therapeutic concentration. The results of the two experiments agreed closely; comparisons with the results of other investigators are made below.

Sodium iron pyrophosphate was poorly available for hemoglobin regeneration, as compared with ferrous sulfate. This is in agreement with Street (1943) and Freeman and Burrill (1945) and in disagreement with Nakamura and Mitchell (1943), who found sodium iron pyrophosphate highly available, i.e., equal to ferric chloride.

Ferric orthophosphate was also poorly available, being significantly inferior to both ferrous sulfate and reduced iron. In a single experiment Freeman and Burrill ranked ferric orthophosphate as slightly less effective than ferric chloride and slightly more effective than reduced iron, although their statistical treatment of the data indicates that these differences were not significant. The explanation for the discrepancy in results is not readily apparent. Since Freeman and Burrill give only the final hemoglobin values at the end of 28 days, a time at which the groups were already well within the normal range of hemoglobin values, it is possible that differences appearing at one to two weeks were no longer discernible. However, it may be pointed out that Day and Stein (1938) found ferric orthophosphate to be much less effective than ferric chloride in preventing a fall of hemoglobin in rats on an iron-deficient purified diet. Furthermore, the clinical studies of Moore, Arrowsmith, Welch, and Minnich (1939) indicated that in man the relatively insoluble ferric orthophosphate, as well as ferrous phosphate, was very poorly absorbed as compared with ferrous sulfate.

Reduced iron was found to be highly available, in agreement with Nakamura and Mitchell and with Freeman and Burrill. It is known to be efficacious clinically in iron therapy.

Ferrous sulfate was found to be highly available, of course, as is well known from its extensive clinical use as the customary form of iron in therapy.

### Summary

The biological availabilities of various types of iron used for bread enrichment have been compared on the basis of hemoglobin regeneration in anemic rats. The iron preparations were fed in the form of enriched breads to groups of 15-19 rats each. The results of two experiments were substantially the same.

Under the conditions of these experiments, both ferrous sulfate and reduced iron were highly available. Ferrous sulfate gave slightly higher experimental values for availability than did reduced iron, but the differences were not statistically significant. However, both ferrous sulfate and reduced iron were significantly more available than ferric orthophosphate and sodium iron pyrophosphate. Ferric orthophosphate appeared slightly more available than sodium iron pyrophosphate, but this superiority was of questionable significance.

The biological availabilities of the various forms of iron were rated approximately as follows with reference to the highly available ferrous sulfate as 100%: ferrous sulfate 100, reduced iron 83, ferric orthophosphate 38, and sodium iron pyrophosphate 21.



These results demonstrate the differences in assimilability of various iron sources and serve to direct attention again to the desirability of using highly assimilable iron sources, such as ferrous sulfate and reduced iron, in bread and flour enrichment, so that the maximum benefit from the iron may be secured by the consumer.

#### Addendum

A more recent investigation at several widely spaced levels of enrichment designed to compare the dosage response curves has demonstrated that, under the conditions of the experiment, ferrous sulfate iron was approximately four to five times as effective as ferric orthophosphate iron in promoting hemoglobin regeneration in anemic rats. Details of the study will be reported later.

#### Acknowledgments

The authors are indebted to Mr. George Garnatz, of the Kroger Food Foundation, Cincinnati, Ohio, for the breads used in these experiments. The authors also wish to express their appreciation to Dr. M. L. Tainter and Dr. L. C. Miller for their interest and criticism, and to Mrs. Mary Lenz for statistical treatment of the data.

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# PURIFICATION OF BARLEY MALT ALPHA-AMYLASE <sup>1</sup>

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Although the starch-degrading action of malt extract stands as a classic historical example of enzyme action, purification of the active principles of malt (alpha- and beta-amylase) has lagged behind similar purification studies on animal and yeast enzymes. Among the many factors contributing to this lag might be mentioned the relative instability of the amylases and, as will be shown later, the relatively small concentrations of these constituents in malt extracts in relation to both the total dry matter and to the total protein present. Furthermore, most early attempts at purification were directed towards the isolation of "malt amylase" preparations (Euler and Svanberg, 1921; Glimm and Sommer, 1927) which, were of course mixtures of alpha- and beta-amylase. More recently Caldwell and Doebbeling (1935) have reported an alpha-amylase preparation which "hydrolyzed 1000 times its weight of starch and formed 337 times its weight of maltose in 30 minutes at 40°." Myrbäck and Örténblad (1941) reported a modification of the procedure of Holmbergh (1938) for preparation of alpha-amylase which resulted in a preparation with a specific activity (on a dry basis) about 20 times that of the starting malt extract. Kneen, Sandstedt, and Hollenbeck (1943) have demonstrated that calcium ions are necessary for the stability of alpha-amylase. They have also presented data on ammonium sulfate and alcohol fractionation, and also on the heat stability of the malt amylases, which indicated the probable utility of these procedures in purification.

The present communication describes procedures for purifying malt alpha-amylase that have been developed by following the protein nitrogen concentrations and enzyme activities of the various fractions. The most highly purified preparations obtained contained about 60% of the activity originally present in the malt extract, and less than 0.5% of the original protein nitrogen. After drying, 1 mg of this preparation is as active (with respect to alpha-amylase) as 5 g of malt.

<sup>1</sup> Enzyme Research Laboratory Contribution No. 106

## Materials and Methods

*Malt Samples Used.* Although a wide variety of malts was used for preliminary work, almost all of the results reported here were obtained with a 100-pound sample of commercial malt containing 7.8% moisture and 2.16% nitrogen (dry basis). The "Maltose Equivalent" of this malt (four times the Lintner value) was equal to 893; the beta-amylase equivalent was 826; and the alpha-amylase equivalent was 67.

TABLE I  
PURIFICATION OF MALT ALPHA-AMYLASE: PROCEDURE A

Step No.	Procedure	Total volume ml	Total units $\times 10^{-4}$	Specific activity	
				Units per mg dry matter (Maltose Equivalent)	Units per mg protein—protein (N $\times 6.25$ )
1	2,000 g ground malt + 15 L 0.1% $\text{CaCl}_2$ , adjust to pH 6.0 with NaOH, stir 2 hours <i>Suspension</i>	15,500	125	67	510
2	Squeeze suspension through cheesecloth; centrifuge extract, discard residue <i>Supernatant</i>	12,500	105	220	2,050
3	Heat supernatant at 70° for 15 minutes, cool to room temperature, filter through layer of 50 g diatomaceous earth (Celite), discard residue <i>Filtrate</i>	12,300	103	300	11,500
4	Ammonium sulfate fractionation; retain fraction between 0.22 and 0.45 saturated, take up residue in 0.1% $\text{CaCl}_2$ <i>Solution</i>	1,000	90	—	46,000
5	Add 25 g bentonite, stir 1 hour, centrifuge, discard residue <i>Filtrate</i>	890	75	—	88,000
6	Ammonium sulfate fractionation; retain fraction between 0.1 and 0.35 saturation, take up residue in 0.1% $\text{CaCl}_2$ <i>Solution</i>	75	70	75,000	105,000
	Repeat steps 5 and 6 <i>Solution</i>	50	60	103,000	163,000

*Determination of Amylase Activities.* When beta-amylase was present, both alpha- and beta-amylase were determined by the methods of Olson, Evans, and Dickson (1944). After the beta-amylase of the malt was destroyed by heating (see Table I, step 3) the alpha-amylase was assayed by a more convenient procedure. To 10 ml of a 2%

buffered solution of soluble starch (0.05 *M* acetate, pH 4.7) were added 5 ml of solution containing 0.5 ml 0.1 *M* calcium chloride solution, enzyme, and water. After 5, 10, or 20 minutes at 30°, depending upon the rapidity of the starch breakdown, 0.5 ml of the digestion mixture was added to 5 ml of a solution of iodine in potassium iodide (0.0035 *M* with respect to iodine and 0.25 *M* with respect to potassium iodide). This hydrolyzate-iodide solution was then diluted by the addition of 10 ml of water, and the light transmission of the diluted mixture at 660  $\mu$  was measured in an Evelyn photoelectric colorimeter at 25°. The relation between percentage transmission and units of alpha-amylase is shown in Figure 1.

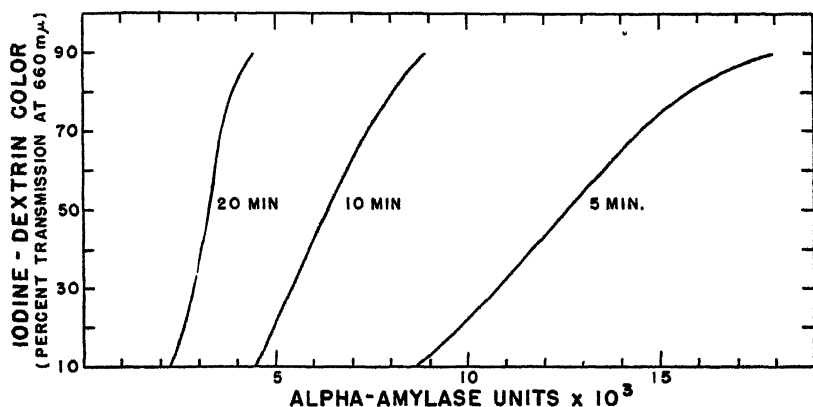


Fig. 1. Relationship between units of alpha-amylase and color change of the dextrin— $I_2$  complex after 5, 10, and 20 minutes incubation, using increasing concentrations of the enzyme with soluble starch under the conditions described in the text.

*Expression of Amylase Units.* The units used in this report describe the amount of alpha-amylase present in the digestion mixture and do not refer to any particular volume or weight of material containing the enzyme. Numerically the units are those employed by Olson, Evans, and Dickson (1944) transformed from a "specific" activity (Maltose Equivalent) to activities which do not in themselves infer the weight of the enzyme preparation. These amylase units per mg of dry weight, as described by Schwimmer (1947), are numerically equal to the "Maltose Equivalent."

*Other Methods Used.* Phosphatase was determined by the liberation of nitrophenol from nitrophenyl phosphate (Axelrod, 1947); phosphorylase by the liberation of phosphate from glucose-1-phosphate by the method of Green and Stumpf (1942); maltase by the method of Schwimmer (1945); proteinase by liberation of tyrosine at pH 6, a modification of the method of Anson (1937); and peroxidase by the delayed oxidation of HI (Schwimmer, 1944). Protein was estimated

by determining the nitrogen content (by micro-Kjeldahl technique) of proteins of the various preparations after precipitation of the proteins by trichloroacetic acid and washing of the precipitate to remove non-protein nitrogen. The reducing action of dextrins and sugars was determined by use of the Somogyi sugar reagent (1945).

## Results

*Extraction and Heating.* Kneen, Sandstedt, and Hollenbeck (1943) have shown that alpha-amylase can be extracted most thoroughly at pH 6.0 in the presence of calcium salts. It has been found that, under aseptic conditions, malt extracts can be kept for two weeks at room temperature without loss of alpha-amylase activity. These conditions are also optimal for stabilizing the alpha-amylase and destroying the beta-amylase of malt extracts when heated to 70° for 15 minutes. Figure 2 depicts the effect of heat on the soluble protein

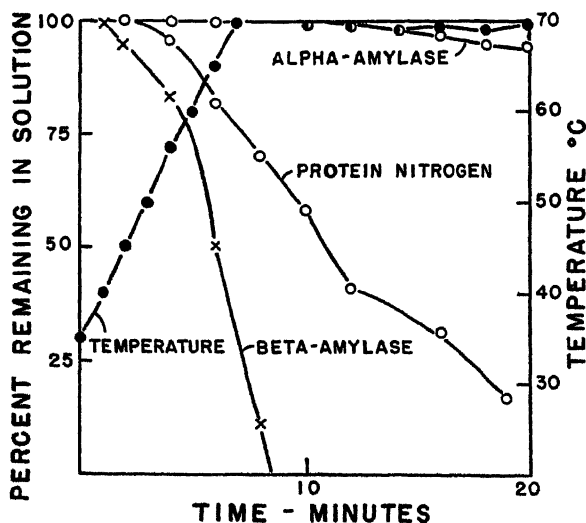


Fig. 2. Changes in protein nitrogen, alpha-amylase, and beta-amylase of a malt extract upon heating. The rise in temperature with time is shown by the temperature curve (referring to the right-hand ordinate); the losses in beta-amylase and protein nitrogen are measured on the left ordinate.

nitrogen and on the alpha- and beta-amylase activities of a malt extract prepared according to steps 1 and 2 of Table I. This heating step not only destroys all of the beta-amylase present in the malt extract but also removes between 75 and 85% of the total protein. It has been observed that when extracts of various malts of equal alpha-amylase and nitrogen contents are heated, those malt extracts with higher beta-amylase values usually precipitate a greater portion of protein than those of low beta-amylase content.

*Ammonium Sulfate Fractionation.* It has been found that the alpha-amylase of malt extracts could be completely precipitated at 0.5 saturation with ammonium sulfate (Caldwell and Doebbeling, 1935; Kneen, Sandstedt, and Hollenbeck, 1943). Figure 3 shows the

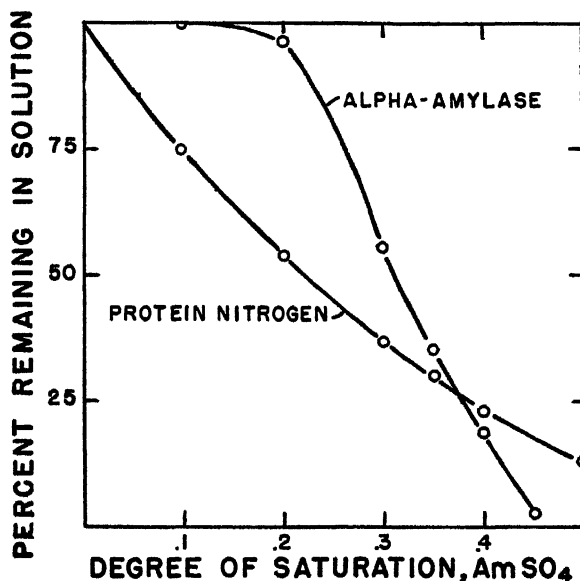


Fig. 3. Effect of adding ammonium sulfate to heated malt extract—protein nitrogen and the alpha-amylase remaining in solution.

percentage of alpha-amylase and protein nitrogen remaining in solution after adding increasing amounts of ammonium sulfate to a heated malt extract. This method of presentation is useful in deciding whether or not a particular fractionation procedure is of any value. Thus, Figure 3 readily shows that if the fractionation is so performed that the upper and lower 5% of the enzyme are discarded, then 70% of the protein will be eliminated, resulting in a threefold increase in specific activity on a protein basis. Further fractionation of this solution is of no obvious advantage since it does not affect the specific activity favorably, as may be seen from Figure 4. However, ammonium sulfate fractionation can be used to advantage at a later stage in the purification (Table I, step 6). The acidity of the protein suspensions varied between pH 5.6 and pH 5.8.

If the freshly prepared malt extract is not treated immediately, but is allowed to remain at room temperature for seven days in the presence of toluene and thymol, the fractionation characteristics with ammonium sulfate are significantly altered. The enzyme is not fully precipitated until 0.65 saturation and the protein precipitation parallels that of the

enzyme rather closely. This change in fractionation properties upon autolysis can possibly be attributed to the change of protein constituents due to proteolysis occurring during the incubation at room temperature.

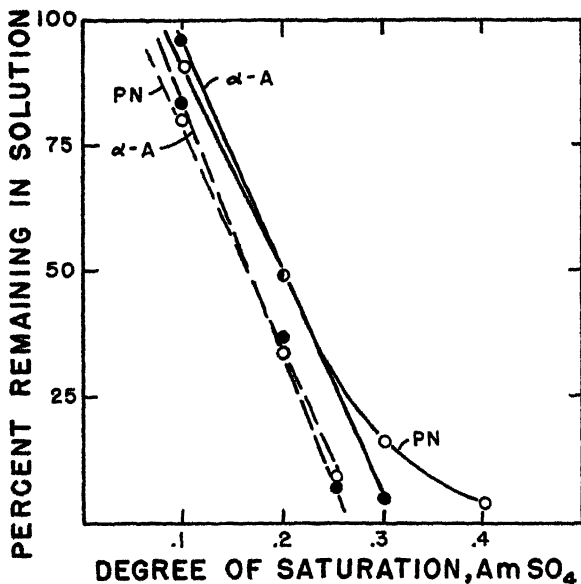


Fig. 4. Protein nitrogen PN (open circles) and alpha-amylase (closed circles) remaining in solution after the addition of ammonium sulfate to (a) once fractionated and heated malt extract (unbroken lines) and (b) twice fractionated and heated malt extract (broken lines).

*Fractionation by Adsorption with Bentonite.* When bentonite (Volclay) was added in increasing amounts to a series of aliquots of the fraction obtained by ammonium sulfate fractionation (Table I, step 4), an appreciable portion of protein and colored impurities could be adsorbed on the bentonite, leaving behind a lightly colored, highly purified solution of alpha-amylase (Figure 5). The adsorption on bentonite is a time reaction, and the success of this step depends upon not allowing the bentonite to stand too long in contact with the solution. It was also found that the adsorption of both protein and enzyme is more complete at lower pHs.

When bentonite was added in a similar manner to the preparations obtained by ammonium sulfate precipitation of heated and incubated malt extracts (Procedure B), no decidedly advantageous increase in specific activity could be obtained. However, if a similar malt extract was treated with bentonite at a concentration of 0.25%, enough protein impurities were adsorbed to increase the specific activity twofold (Table II, step 4). Furthermore the unadsorbed enzyme could now

be adsorbed by adding further amounts of bentonite and then, subsequently, could be eluted from the bentonite with a 5% pyridine solution containing 0.1%  $\text{CaCl}_2$ .

*Use of Other Fractionation Procedures.* During the course of these investigations, many fractionation procedures were tried, but no one was entirely satisfactory. Precipitation with lead acetate or sodium chloride gave variable results. The use of organic solvents and of acid pHs, while precipitating much protein, rendered the enzyme extremely unstable. Use of nucleic acid (Butler, 1945), kaolin (Sabalitschka and Weidlich, 1929) and starch granules (Holmbergh, 1933) as adsorbents proved unsatisfactory.

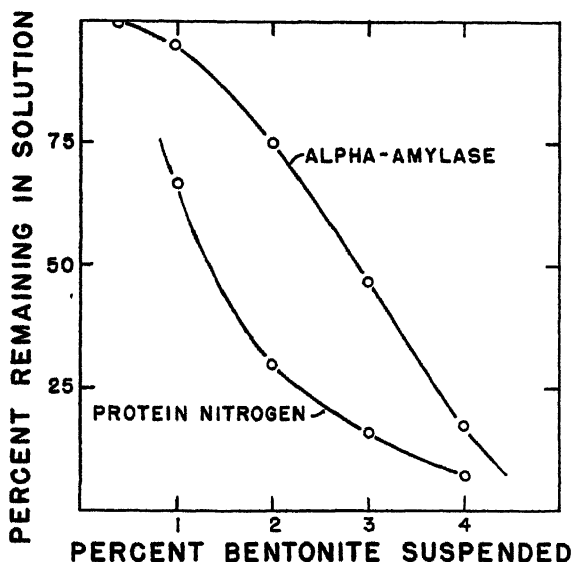


Fig. 5. Adsorption of protein and alpha-amylase by increasing concentrations of bentonite added to malt extract previously heated and fractionated with ammonium sulfate. The volume of this fraction is 1/15 that of the original malt extract. Percent bentonite is equal to grams bentonite suspended in 100 cc of solution.

*Properties of Final Preparations.* The two procedures adopted as standard are summarized in Tables I and II. Method A, which preceded Method B chronologically, gave a preparation which was light brown in color, gave a positive Molisch test for carbohydrate, and contained 10.6% protein nitrogen (64% protein). This preparation, which exhibited reducing action when tested with Somogyi's copper reagents (1945), increased its reducing action three- to fourfold when hydrolyzed with 3 *N* HCl. This may indicate the presence of reducing dextrans of low molecular weight in association with the protein. These dextrans could be eliminated by allowing the malt extract to



TABLE II  
PURIFICATION OF MALT ALPHA-AMYLASE: PROCEDURE B

Step No	Procedure	Total volume ml.	Total units $\times 10^{-4}$	Specific activity	
				Units per mg dry matter (Maltose Equivalent)	Units per mg protein—protein (N $\times 6.25$ )
1	2,000 g ground malt + 15 L 0.1% $\text{CaCl}_2$ , adjust to pH 6.0 with NaOH, stir 2 hours <i>Suspension</i>	15,500	125	67	510
2	Squeeze suspension through cheese cloth, centrifuge extract. Allow supernatant to stand at room temperature for 7 days <i>Supernatant</i>	12,500	105	220	2,230
3	Heat supernatant at 70° for 15 minutes; cool to room temperature, filter through layer of 50 g diatomaceous earth <i>Filtrate</i>	12,300	104	310	13,600
4	Add 30 g bentonite, stir 30 minutes, centrifuge, discard residue <i>Supernatant</i>	12,000	90	—	29,100
5	Add 30 g bentonite, stir 30 minutes. Centrifuge, discard supernatant. Residue + 1 L 5% pyridine containing 0.1% $\text{CaCl}_2$ ; let stand at room temperature 2 hours, centrifuge, discard residue <i>Supernatant</i>	1,000	81	—	85,000
6	Add 225 g ammonium sulfate (0.37 saturated), centrifuge, discard supernatant; take up residue in 200 ml 0.1% $\text{CaCl}_2$ <i>Supernatant</i>	200	71	356,000	360,000

autolyze at room temperature for seven days in the presence of thymol and toluene. Such autolysis probably allowed both the alpha- and beta-amylase to completely hydrolyze any soluble dextrin and starch present in the extract. At any rate the pure preparations made by Procedure B contained between 16 and 16.5% nitrogen and gave negative or very doubtful positive Molisch tests. These preparations were somewhat more highly colored than those made by Method A. Their light absorption was determined by means of a Beckman spectrophotometer. There were no characteristic absorption peaks throughout the visible spectrum. The usual absorption by protein at 280  $m\mu$  was partially masked by a general absorption probably due to the highly colored impurities present (Figure 6).

The enzymic properties of the preparations corresponded to those generally attributed to malt alpha-amylase. The enzyme did not

dialyze through cellophane, and was inactivated and rendered insoluble when dialyzed against distilled water. This inactivation could be prevented by either dialyzing against 0.05% calcium chloride solution or by placing an excess of calcium sulfate in the dialysis bag. Repeated precipitation with ammonium sulfate also resulted in a water-insoluble and inactive material unless calcium chloride solution

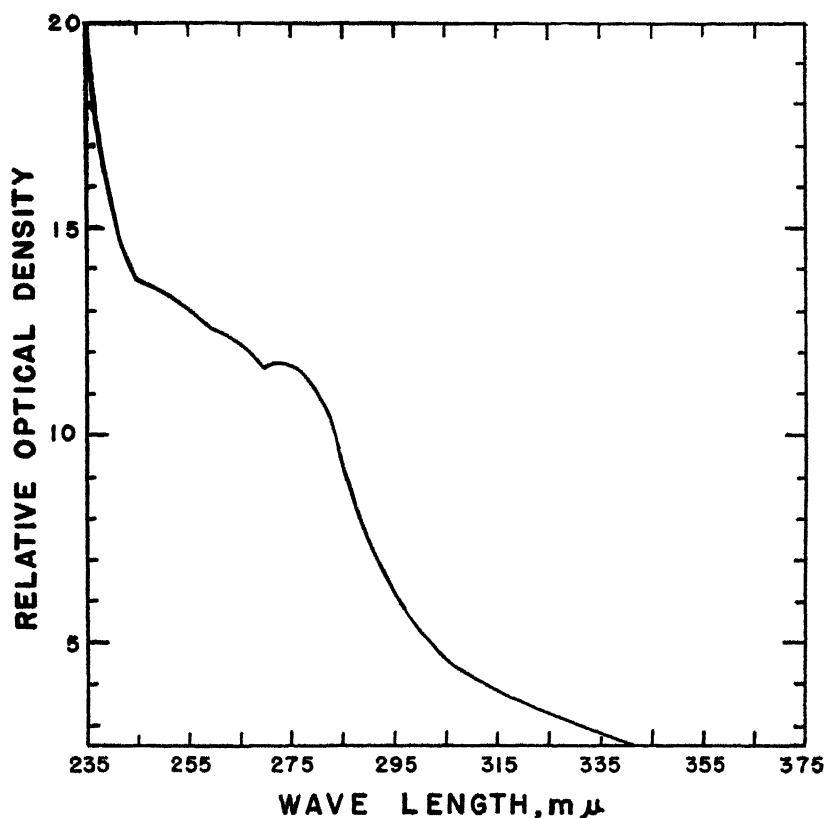


Fig. 6. Ultraviolet absorption spectrum of Preparation B6.

was used to dissolve the precipitates. Sodium tetraphosphate ( $\text{Na}_4\text{P}_4\text{O}_{13}$ ) inactivated the enzyme when incubated at pH 5.0 in concentrations high enough to tie up the calcium in the preparations. Sodium phosphate under the same conditions of acidity and temperature did not affect the alpha-amylase activity. The dependence of the amylase stability on the presence of calcium ion is in accordance with the observations of Kneen, Sandstedt, and Hollenbeck (1943). In agreement with the observation of Caldwell and Doebbeling (1935),

the purified enzyme is much more sensitive to heat than when present in the crude extract.

The final preparation (B6) contained 67% of the total alpha-amylase of the malt extract and 0.4% of the original extract protein. If the protein in this preparation represents only pure enzyme protein, the maximum concentration of alpha-amylase in the malt extract cannot amount to more than 0.06% of the dry weight nor more than 0.6% of the protein of the extract.

Alpha-amylase preparations prepared by either method were found to be devoid of the following enzyme activities: beta-amylase, maltase, proteinase, peroxidase, and phosphorylase. They contained a maximum of 0.02% of the original acid phosphatase.

### Summary

Purification studies on the alpha-amylase of malt extracts have led to preparations which contain 60–70% of the alpha-amylase, 0.4% of the protein, and about 0.05–0.08% of the dry matter of the starting malt extract. This amounts to a 150-fold purification on a protein basis and about 1,500-fold purification on a dry weight basis. The Maltose Equivalent ( $4 \times ^\circ\text{L}$ ) was about 300,000. One milligram of the preparation is as active as 5 g of malt. Each possible purification step attempted was followed by estimating the protein nitrogen as well as the alpha-amylase content of the various fractions obtained by varying the concentration of the fractionating agent used.

The purification procedure which has yielded the most potent preparations consists (in brief) of: autolysis of the malt extract, followed by adsorption of impurities on bentonite; adsorption of the alpha-amylase by further addition of bentonite; elution of the enzyme with pyridine solution; fractionation of the eluate with ammonium sulfate.

The stability and hydrolytic properties of the purified alpha-amylase are in agreement with those properties generally attributed to alpha-amylase.

### Acknowledgments

Grateful acknowledgment is made to Dr. A. K. Balls of the Enzyme Research Laboratory for helpful suggestions during the course of this work. The author also wishes to thank G. F. Bailey of the Western Regional Research Laboratory for the ultraviolet absorption data and Dr. A. Frieden of the Pabst Brewing Company of Milwaukee for his generous gift of barley malt.

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## AMYLASE ACTIVITY OF THREE BARLEY VARIETIES AS INFLUENCED BY DIFFERENT MALTING CONDITIONS <sup>1</sup>

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This report represents a continuation of previous studies of the changes taking place in the barley kernel during the malting process (Shands *et al.*, 1941; Shands *et al.*, 1942; and Dickson and Burkhart, 1942). These earlier studies did not include data on the influence of malting conditions upon the development of the individual amylase components. Therefore, emphasis is given here to the formation of alpha- and beta-amylase in three samples of barley malted under different conditions of moisture, time, and temperature.

<sup>1</sup> Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, A.R.A., U.S.D.A., Wisconsin Agricultural Experiment Station, United States Maltsters Association, and Malt Research Institute.

Competent reviews of the literature on the effects of various factors upon the development of the amylases in the course of germination of cereals are given by Weichherz and Asmus (1931), Lüers (1936), and more recently by Kneen, Miller, and Sandstedt (1942). Anderson and Sallans (1938), Anderson and Meredith (1938), and Sallans and Anderson (1939) in studies of varietal differences of barley have reported on the influence of moisture, temperature, and germination time on diastatic power.

In earlier studies by Shands *et al.* (1941) using constant malting temperatures, and Shands *et al.* (1942) using systematically changed temperatures during malting, it was shown that several quality factors were affected besides the diastatic enzyme system of barley. Reference should be made to these papers for information relating to the other quality factors.

Shands *et al.* (1941) found a close relationship between factors influencing acrospire growth and diastatic power. They also reported that high malting moisture and increased malting time favored the development of diastatic power. The increase in diastase was greatest in the early phases of malting, and was less from 6 to 8 days under most of the conditions employed. Although all varieties studied showed a common trend, the magnitude of the changes differed between varieties. In later studies (1942), these workers found the maximum average diastatic power was obtained in a temperature change schedule during malting involving 12° and 16°C and 6 days total germination. Moreover, it was found that higher diastatic power usually accompanied higher wort and formol nitrogen ratios, and shorter conversion times.

In studies relating to the distribution of diastatic power in the barley and malt kernel, Dickson and Burkhart (1942) found the bulk of the increased activity was formed between the second and sixth day of malting. Higher malting moisture (48%) gave uniformly higher values than did the lower moisture (43%) samples. Increase in activity occurred only in the germ portion of the kernel, the distal half remaining essentially unchanged after the fourth day of malting. The results of these workers on alpha-amylase development were essentially the same as those reported for diastatic power.

### Methods and Materials

Samples of Wisconsin Barbless (C.I. 5105) from Michigan, Wisconsin hybrid H35-7-2-1-3 (C.I. 7115) grown at Madison, Wisconsin, and Peatland (C.I. 5267) obtained from St. Paul, Minnesota, all grown in the 1943 crop year, were chosen for this study. The Wisconsin Barbless was low, Wisconsin H35-7-2-1-3 intermediate, and Peatland

high in amylase activity. The nitrogen contents of the barleys used were: Wisconsin Barbless, 1.92%; Wis. H35-7-2-1-3, 2.32%; and Peatland, 2.61%. Since these barleys were chosen for their ranges in amylase values, the responses of the samples to malting conditions cannot be attributed solely to variety.

The barleys were cleaned, steeped in water at 16°C in screen-bottomed cans 5 inches in diameter and 3 inches high to either 45%, or 48% moisture, and the steeped barleys germinated for 4, 6, 8, or 10 days in the small experimental unit previously described by Shands *et al.* (1942) at 12°C, 16°C, 20°C, or under the conditions of temperature change employed, i.e., 2 days at 20°C and the remaining 8 days at 12°C. At the end of the indicated germination times, the green malts were subjected to a uniform kilning procedure, namely: 8 hours at 25°C, 4 hours at 35°C, and 20 hours at 45°C. The kiln used by Shands *et al.* (1941) was employed in the drying of these malts. The dried samples (about 6.3% moisture) were freed of rootlets, and the cleaned malts were analyzed by standard methods.

Diastatic activity was determined by the alkaline ferricyanide method described in *Cereal Laboratory Methods*, A.A.C.C., 1941. Alpha-amylase was determined by the method of Sandstedt, Kneen, and Blish (1939) as modified for 20°C by Olson, Evans, and Dickson (1944). The activity is expressed in 20°C dextrinizing units as described by these authors in a recent communication (1947). Beta-amylase was calculated from the alpha-dextrinizing activity<sup>2</sup> and the diastatic activity in the customary manner (Olson, Evans, and Dickson, 1944).

In addition to the amylolytic enzyme values, extract, wort, and formol nitrogen, kernel weight, growth, and other values reported by Shands *et al.* (1941, 1942) were obtained. These values are not discussed in detail here, inasmuch as the effects of the various treatments are in agreement with those reported previously.

## Results

The four variants, time, temperature, moisture, and sample malted, affected the amylase content of the malts produced. The influence of each of these on the amylase contents of the malts will be discussed. The results of the amylase determinations made upon the malts produced as indicated above are presented in Table I and in Figures 1 to 3.

*Effect of Malting Conditions on Diastatic Power.* The influence of germination conditions on the diastatic power of the malts produced in this study is represented graphically in Figure 1. The higher (48%)

<sup>2</sup> The terms "alpha-amylase" and the more strictly correct "alpha-dextrinizing activity" will be used interchangeably in this discussion.

malting moisture usually resulted in increased diastatic power in the three barley samples studied. Malts produced from Peatland barley at 48% moisture under the conditions of temperature change during germination showed lower diastatic activity than those malted under

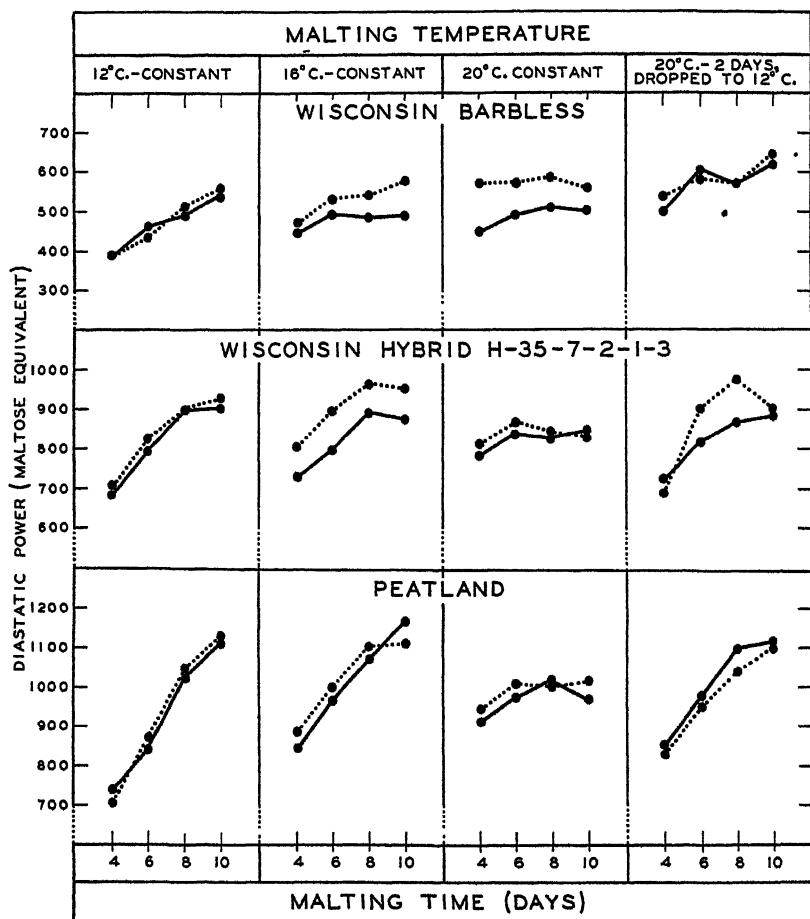


Fig. 1. The effect of malting time and temperature upon diastatic power of malts produced at 45% (—) and 48% (.....) moisture.

the same conditions at 45% moisture. The influence of moisture upon diastatic power was not as great at the 12°C as at the 16°C malting temperature. Germination at 48% malting moisture and 20°C resulted in malts of higher diastatic activity in the Wisconsin Barbless malts; this effect of moisture at this temperature was not as apparent in the other two samples studied.

A general increase in diastatic activity with increased malting time through the eighth day of germination was apparent in the malts produced at 12°C, 16°C, and in the temperature change series. The changes in diastatic activity from the eighth to tenth day of germination were unpredictable, and the increase or decrease in activity was dependent on sample malted and temperature. However, Peatland and Wisconsin Barbless germinated at 12°C showed significant increases with time. Peatland diastase also increased between 8 and 10 days at 45% moisture and 16°C. The bulk of the diastatic power in the malts germinated at 20°C apparently had been formed in the first 6 days of germination; the changes in activity thereafter were slight.

The diastatic activity of the 4-day malts increased as the temperature of malting was raised through 20°C, while the activity of the 4-day malts germinated under the temperature change conditions simulated those produced at either 12°C, or 16°C. Final diastatic power values were usually lower when the barleys were malted at 20°C than they were at the other temperatures. Malting at 12°C and under the temperature change schedule yielded malts of approximately equal final diastatic activity.

*Effect of Malting Conditions on Beta-Amylase.* The statements made above on the influence of malting conditions upon diastatic activity apply to beta-amylase development, as can be seen in Figure 2. The values are somewhat lower because alpha-amylase saccharifying activity has been removed from the diastatic power values. However, a few observations can be made which were masked in the diastatic activity measurements. The final level (10-day malt) of beta-amylase decreased with an increase in malting temperature in the Wisconsin hybrid H35-7-2-1-3, and Peatland malts, if averages of the two moisture levels are considered. There is little effect of malting temperature upon the final value of beta-amylase in the Wisconsin Barbless malts, except that the high moisture malts had higher beta-amylase than those produced under the low moisture conditions. A loss of beta-amylase activity at 20°C and high moisture was observed in the Wisconsin hybrid H35-7-2-1-3 and Peatland malts following the sixth day of germination, and in the Wisconsin Barbless malts following the eighth day of germination at 20°C. At 16°C, the Wisconsin hybrid malts showed a decrease in beta-amylase from 8 to 10 days growth.

It should be pointed out here that the loss of beta-amylase activity is accentuated somewhat when the results are calculated to the basis of the original barley used (including the dry matter lost in respiration, cleaning, etc.). However, the trends presented in this report would not be altered materially by the recalculation.



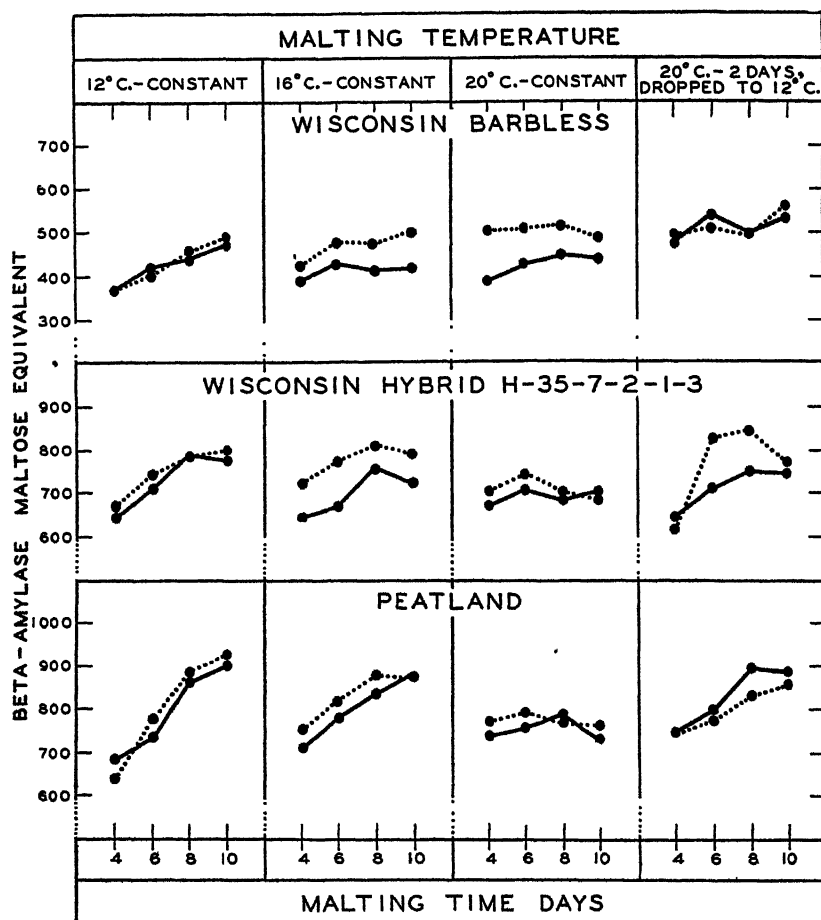


Fig. 2. The effect of malting time and temperature upon beta-amylase activity of malts produced at 45% (—) and 48% (.....) moisture.

*Effect of Malting Conditions on Alpha-Dextrinizing Activity.* The development of alpha-dextrinizing activity in the course of germination under the experimental conditions employed is presented in Figure 3. Malting moisture seemed to have little, if any, effect upon alpha-amylase development in this experiment.

There was a general increase in alpha-amylase with longer germination times, and the rate of increase was less at the higher temperatures. There was a marked increase in alpha-amylase between 4 and 8 days growth in H35-7-2-1-3 and Peatland malts. At the 4 and 6 day stages of germination, an increase in malting temperature resulted in increased alpha-dextrinizing activity in the Wisconsin hybrid H35-7-2-1-3

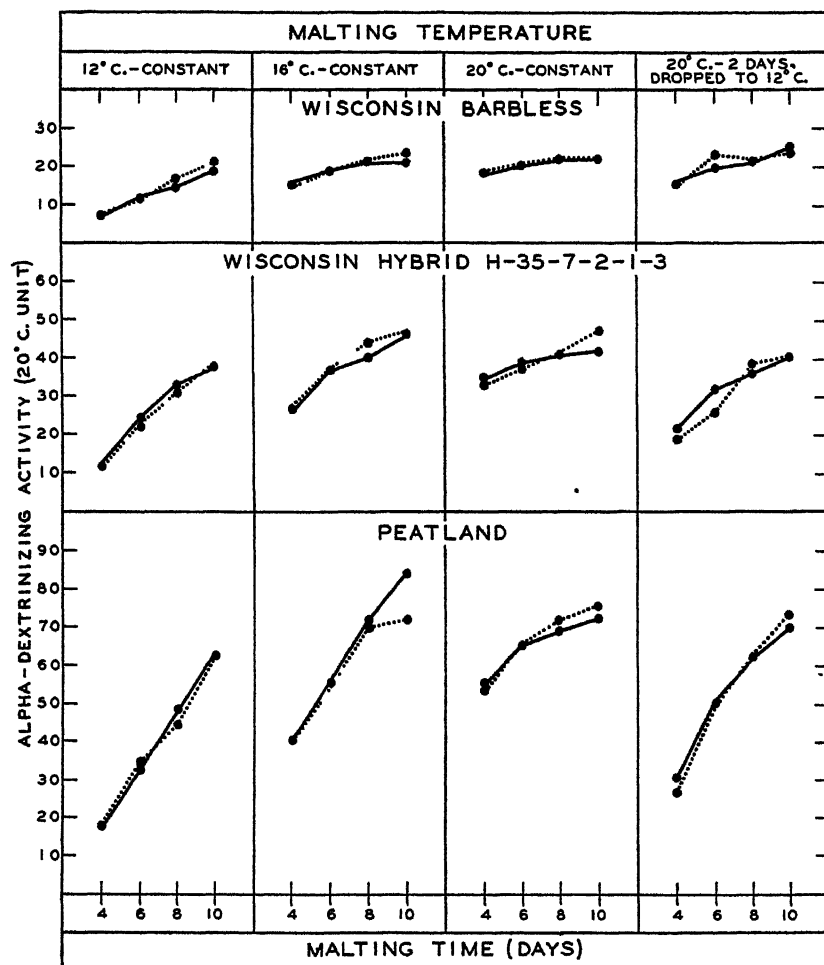


Fig. 3. The effect of malting time and temperature upon alpha-dextrinizing activity of malts produced at 45% (—) and 48% (....) moisture.

and Peatland malts. There was little effect of malting temperature above 16°C upon the 8 and 10-day malts except for Peatland at 45% moisture. The 12°C malts were lower in activity throughout. As might be expected, malts produced by the temperature change schedule assumed a position intermediate between the 12°C and 16°C series for Wisconsin hybrid H35-7-2-1-3 and Peatland, but the Wisconsin Barbless malts produced under these conditions had activities similar to those malted at 16°C.

*Effect of Malting Conditions on the Beta-Amylase Alpha-Amylase Ratio.* The ratios of beta- to alpha-amylase are presented for all

treatments in Table I. While ratios should be used with care, they do show certain interesting trends. The rapid decrease in this value with time, particularly at 12°, emphasizes the difference in time of production or activation of the two components. Comparison of the 4-day values for the different temperatures within any one variety shows that the production of alpha-amylase is increased more with an increase in temperature than is beta-amylase. The lowest ratios were

TABLE I  
INFLUENCE OF THE TEMPERATURE AND TIME OF MALTING AT  
45% AND 48% MOISTURE ON THE AMYLASE CONTENT OF  
THE MALTS OF THREE BARLEY SAMPLES

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
WISCONSIN BARBLESS						
12°C—constant	45	4	384	366	6.8	53.8
	45	6	460	422	12.3	34.3
	45	8	492	444	14.9	29.8
	45	10	544	480	19.0	25.3
	48	4	384	366	6.9	53.0
	48	6	440	405	11.7	34.6
	48	8	516	459	17.2	26.7
	48	10	560	488	21.0	23.2
16°C—constant	45	4	444	391	16.0	24.4
	45	6	496	431	19.3	22.3
	45	8	488	416	21.2	19.6
	45	10	492	420	21.3	19.7
	48	4	472	424	14.8	28.6
	48	6	536	481	18.9	20.4
	48	8	544	478	22.1	21.6
	48	10	580	506	23.6	21.4
20°C—constant	45	4	448	387	18.3	21.1
	45	6	492	431	20.4	21.1
	45	8	516	450	22.1	20.4
	45	10	508	441	22.2	19.9
	48	4	572	508	19.0	26.7
	48	6	576	511	21.6	23.7
	48	8	588	521	22.4	23.3
	48	10	560	491	22.6	21.7
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	500	446	16.3	27.4
	45	6	604	537	20.0	26.9
	45	8	572	500	21.2	23.6
	45	10	624	537	25.4	21.1
	48	4	540	492	14.9	33.0
	48	6	584	513	23.4	21.9
	48	8	572	496	22.0	22.5
	48	10	648	566	23.8	23.8

TABLE I—*Continued*

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
WISCONSIN HYBRID H35-7-2-1-3						
12°C—constant	45	4	680	641	12.2	52.5
	45	6	792	710	24.9	28.5
	45	8	900	790	33.1	23.9
	45	10	904	777	37.8	20.6
	48	4	700	667	11.2	59.6
	48	6	824	743	23.4	31.8
	48	8	904	784	31.0	25.3
	48	10	928	800	38.4	20.8
16°C—constant	45	4	728	646	26.0	24.8
	45	6	796	672	37.1	18.1
	45	8	896	758	40.7	18.6
	45	10	880	722	46.2	15.6
	48	4	804	718	27.4	26.2
	48	6	896	772	37.2	20.8
	48	8	964	812	44.3	18.3
	48	10	952	790	47.2	16.7
20°C—constant	45	4	784	669	34.8	19.2
	45	6	840	710	38.8	18.3
	45	8	828	685	41.8	16.4
	45	10	848	705	42.4	16.6
	48	4	812	702	33.2	21.1
	48	6	868	743	37.3	19.9
	48	8	844	701	42.0	16.7
	48	10	832	684	47.5	14.4
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	720	645	21.9	29.5
	45	6	820	713	30.2	23.6
	45	8	872	753	36.2	20.8
	45	10	884	746	40.7	18.3
	48	4	684	616	19.9	31.0
	48	6	908	825	26.4	31.3
	48	8	976	844	39.1	21.6
	48	10	904	766	40.9	18.7
PEACELAND						
12°C—constant	45	4	736	679	17.2	39.5
	45	6	844	736	32.8	22.4
	45	8	1032	864	47.8	18.1
	45	10	1112	902	63.7	14.2
	48	4	708	633	17.8	35.6
	48	6	868	745	34.2	21.8
	48	8	1048	890	45.7	19.5
	48	10	1132	927	62.4	14.9

TABLE I—*Continued*

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
PEATLAND						
16°C—constant	45	4	844	710	40.2	17.7
	45	6	968	782	58.2	13.4
	45	8	1072	835	71.8	11.6
	45	10	1168	882	84.6	10.4
	48	4	884	750	40.0	18.8
	48	6	1000	826	55.0	15.0
	48	8	1108	877	70.0	12.5
	48	10	1112	873	72.3	12.1
20°C—constant	45	4	912	740	55.0	13.5
	45	6	976	758	65.6	11.6
	45	8	1020	790	69.3	11.4
	45	10	968	729	72.5	10.1
	48	4	944	773	53.8	14.4
	48	6	1012	793	66.0	12.0
	48	8	1004	767	72.0	10.7
	48	10	1016	763	75.8	10.1
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	853	752	30.8	24.4
	45	6	976	800	51.2	15.6
	45	8	1100	895	62.3	14.4
	45	10	1116	886	70.0	12.7
	48	4	828	743	27.0	27.5
	48	6	948	776	49.8	15.6
	48	8	1040	832	63.0	13.2
	48	10	1104	857	73.5	11.7

produced at 20° by the Wisconsin hybrid and Peatland Malts, and at 16° in Wisconsin Barbless Malts.

Malts produced from Wisconsin Barbless and Wisconsin hybrid H35-7-2-1-3 show approximately the same proportion of the two components, although the latter is much higher in activity. The Peatland malts are highest in activity and show a lower ratio of beta- to alpha-amylase under all conditions.

### Discussion

It should be emphasized that the conditions of malting employed in these studies are in many instances completely unsuitable for commercial operations, and the malts prepared by the use of some of the methods would not be acceptable to the industry. The malting losses, particularly at 20°C, were often above a level considered economically feasible by the maltster. Increasing amylase activity by the methods described herein may be disadvantageous because of the long times

involved at the low temperatures or the high malting losses at the higher temperatures. Therefore, the results of these experiments should not be considered directly applicable to commercial malting, but rather as studies designed to understand more completely the physiology of activation of the amylases accompanying malting.

The differential effects of malting moisture upon the development of the two amylase components are of considerable interest. Both diastatic power and beta-amylase were significantly higher when the barleys were germinated at high (48%) moisture. This moisture effect is in agreement with earlier observations of Shands *et al.* (1941), of Dickson and Burkhardt (1942), and of Sallans and Anderson (1939). Alpha-amylase was similar for both moistures used. Dickson and Burkhardt (1942) found a significant increase in alpha-amylase activity accompanying higher (48%) malting moisture. It is significant to note that the low moisture level used by Dickson and Burkhardt was 43%, or 2% lower than that used in the present study. It would appear from this that moistures below 45% may affect the production of alpha-amylase. At moistures above 45% there is little moisture effect. This observation requires further experimental confirmation using a larger number of barleys.

The length of germination time was an important factor in the development of amylase activity. As might be expected, the time effect was more noticeable at the lower malting temperatures, where growth was slower. Anderson and Sallans (1938) showed that diastatic power increased at 50°F through 13 days of malting but reached a maximum value after 9 days at 56°F. The differential response of samples to germination time with respect to rate of beta-amylase production is in harmony with the observations of Weichherz and Asmus (1931), and Anderson and Meredith (1938). An actual loss of beta-amylase activity at 20°C and high moisture was observed in some of the samples studied. Loss of beta-amylase activity on germination has been observed by Kneen, Miller, and Sandstedt (1942) with wheat.

Alpha-amylase activity increased with time of germination, irrespective of variety or temperature of malting, although Wisconsin Barbless had low activity and was not very responsive. There was also little effect of temperature of malting above 16°C on the final yield of alpha-dextrinizing activity. It would have been interesting to extend germination under these conditions to determine whether or not definite temperature effects would have shown up at later stages in the Wisconsin Barbless Malts.

Higher malting temperatures have for the most part increased initial alpha-amylase activities in the malts, and, as mentioned pre-

viously, decreased beta-amylase content, particularly in the later stages of germination. In the main, the temperature change series malts assumed a position between the 12°C and 16°C series, but resulted in increased yield of beta-amylase in the high moisture Wisconsin hybrid malts at 6 and 8 days germination, and in all the Wisconsin Barbless malts.

It is felt the present study represents an approach to more detailed studies of the physiology of amylase development accompanying the malting of barley under controlled conditions, but the results obtained must be considered as preliminary. The malting moisture effects require further study as indicated. An understanding of variation caused by the barley variety and the environment prevailing during the growing season in relation to the response of the barley to malting would be of value.

### Summary

Individual samples of three varieties of barley, Wisconsin Barbless, Wisconsin hybrid H35-7-2-1-3, and Peatland were each malted under different conditions of moisture, temperature, and time. Four malting temperature schedules (12°, 16°, and 20°C constant, and 2 days at 20°C then dropped to 12°C), two malting moistures (45 and 48%), and four germination times (4, 6, 8, and 10 days) were used, giving a total of 32 combinations.

The influence of the various malting conditions on the development of the amylase components is reported. Time of germination was the most important single factor in the development of alpha-amylase activity. Malting temperatures above 16°C resulted in more rapid development of alpha-amylase, but affected the final values only slightly. Alpha-amylase was usually unaffected by malting moisture.

In general, maximum beta-amylase activity was obtained more rapidly as the temperature of malting increased. At 20°C, and occasionally at 16°C, further malting resulted in malts of somewhat lower beta-amylase activity. Beta-amylase values were usually higher in malts germinated at the higher moisture.

### Acknowledgments

The assistance of Gordon Grimm in the malting of the samples, Mrs. Mary Alexander in the analytical work, and Eugene H. Herrling in the preparation of the graphs is greatly appreciated.

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The first tests were made on 6% suspensions of various kinds of starch with and without 2.0% (dry basis) polyoxyethylene stearate added to the suspension before heating. Four hundred grams of each suspension were heated in the amylograph at the rate of 1.5°C per minute from 40°C until maximum viscosity was obtained and transferred to glass jars as soon as the viscosity started to decrease. The starch pastes were stored overnight at room temperature and then 300 g returned to the machine and mixed for 10 minutes at 30°C. The maximum hot viscosity and the minimum viscosity of these pastes the next day (multiplied by the factor 1.33 to correct for the smaller charge) are shown in Table II.

TABLE II  
EFFECT OF POLYOXYETHYLENE STEARATE ON THE VISCOSITY  
OF VARIOUS KINDS OF STARCH PASTE

Starch	Maximum hot viscosity			Minimum cold viscosity, next day <sup>1</sup>		
	Control	+2% Ester	Change	Control	+2% Ester	Change
	BU <sup>2</sup>	BU	%	BU	BU	%
Wheat	50	70	+40	466	207	-56
Corn	170	220	+30	1080	500	-54
Tapioca	370	360	-3	760	427	-43
Waxy corn	600	485	-19	587	507	-14
Potato	610	440	-28	1333	563	-58

<sup>1</sup> Minimum viscosities were computed from the actual values by multiplying by the factor 1.33 to compensate for the smaller charge used as compared with that taken in determining maximum hot viscosities.

<sup>2</sup> Brabender units.

The ester increased the hot paste viscosities of wheat and corn starch, had no effect on tapioca starch, and decreased that of waxy corn and potato starch. All viscosity readings taken after 10 minutes' mixing the next day and corrected to 400 g show an increase over the maximum when made, except for the waxy corn control. However, on the next day the pastes containing the ester show a significant decrease in viscosity when compared to the controls of the same age. This decrease is not great for the waxy corn, but it is around a 50% reduction for the others, which is certainly significant and of the same order for the four kinds of starch irrespective of their comparative gel strength or viscosity. These tests therefore indicated that it was this ability of polyoxyethylene stearate to reduce the viscosity or "set-up" of starch pastes after standing, that was responsible for its effect on bread crumb.

The effect of smaller amounts of ester on starch paste viscosity was shown by preparing a series of 6% corn starch pastes containing 0, 0.5, 1.0, and 2.0% of the ester and treating them as described above.



TABLE III  
EFFECT OF CONCENTRATION OF POLYOXYETHYLENE STEARATE ON  
VISCOSITY OF 6% CORN STARCH PASTE

Ester <sup>1</sup>	Temperature at max. visc.	Viscosity in Brabender units		Reduction in viscosity
		Maximum-hot	Minimum-cold (next day) <sup>2</sup>	
%	°C	BU	BU	%
0	88.0	170	1080	—
0.5	92.5	210	665	38
1.0	93.0	230	558	48
2.0	93.0	220	500	54

<sup>1</sup> Concentration is expressed as % of dry starch.

<sup>2</sup> Values in Figure 3  $\times 1.33$ .

Since the apparent temperature of gelatinization increased with increasing amounts of ester, these and the viscosity data are shown in Table III and the curves themselves in Figure 3.

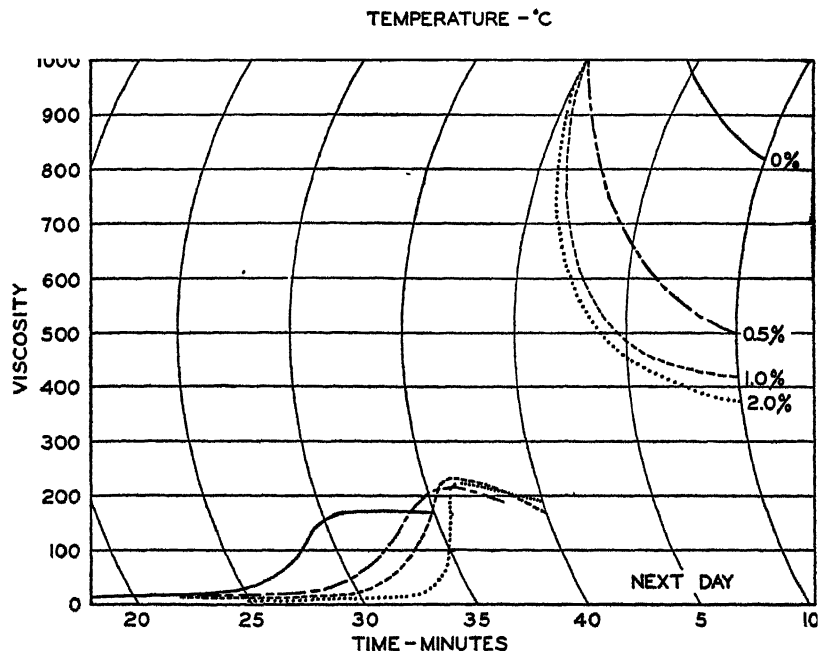


Fig. 3. Effect of concentration of polyoxyethylene stearate on viscosity of 6% corn starch paste.

The increase in apparent temperature of gelatinization is similar to that obtained when the concentration of starch is reduced (Anker and Geddes, 1944) and may well be due to a wetting or lubricating action of the ester. Maximum viscosities increase somewhat as the

concentration of ester increases but do not appear to be particularly significant. It is the progressive decrease in the viscosity of the pastes when remixed the next day that is outstanding and proves that this decrease is truly a function of the additive.

In order to determine whether this material actually affected the progressive setting up or retrogradation of starch paste with further aging, a larger batch of starch was required so that aliquots of paste which had not been disturbed since they were cooked might be measured in the amylograph. Two kilogram batches of 6% corn starch with 0.0, 0.5, and 1.0% (dry basis) polyoxyethylene stearate added were cooked in a 5-quart Merryway cake mixing bowl surrounded by

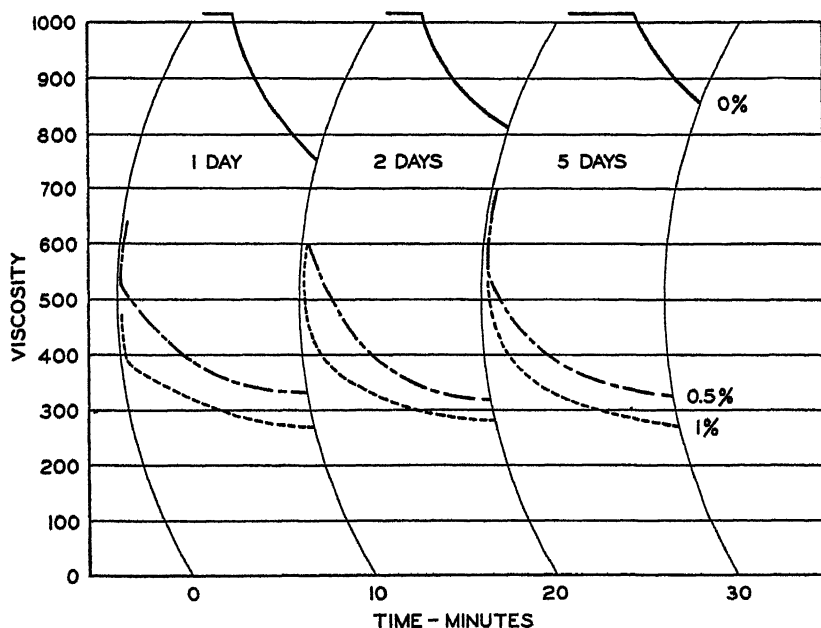


Fig. 4. Effect of polyoxyethylene stearate on the change in viscosity of 6% corn starch pastes with age.

a boiling water bath and with the paddle turning at low speed during the entire cook. Heating for one hour produced a paste temperature of only 90°C, so this paste was not a duplicate of those made in the amylograph and an effect of incomplete gelatinization would be expected in the pastes containing the ester. However, conditions between batches could be held constant and the viscosities obtained were of normal magnitude. To prevent spoilage, 2 ml of formaldehyde solution were added just before each batch was packed in sealed pint jars, and these were then cooled and stored at room temperature.

After one, two, and five days' storage a 300 g charge from each batch was transferred to the amylograph, warmed to 30°C, and run for 10 minutes. The curves obtained and the data taken from them are shown in Figure 4 and Table IV. These show the progressive increase

TABLE IV  
EFFECT OF POLYOXYETHYLENE STEARATE ON THE CHANGE IN  
VISCOSITY OF 6% CORN STARCH PASTES WITH AGE

Ester	Minimum cold viscosity after 10 minutes mixing <sup>1</sup>		
	1-day-old	2-days-old	5-days-old
%	<i>BU</i>	<i>BU</i>	<i>BU</i>
0.0	1020	1065	1150
0.5	445	440	440
1.0	372	378	368

<sup>1</sup> Values in Figure 4  $\times 1.33$ .

in the viscosity of starch pastes with age and the ability of polyoxyethylene stearate to prevent or mask it.

### Summary

Polyoxyethylene stearate was found to have a pronounced retarding effect on the development of firmness of bread crumb with age. In quantities up to 2.0% of the flour weight, this material had no significant effect on bread doughs during their processing or on the physical character of the bread other than its softness and tenderness. Baking tests supplemented by farinograph and pressuremeter tests showed that there was no effect on the condition of the gluten or the rate and amount of gas produced by the yeast.

Addition of polyoxyethylene stearate to starch pastes increased the apparent temperature of gelation but did not prevent complete gelatinization at the higher temperature. Pastes containing it showed much less "set-back" or stiffening after cooling and storage for one day and no tendency to increase in viscosity or retrograde with more extended aging.

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## NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS<sup>1</sup>

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Corn, which is called by some the imperial agricultural plant of America, is of great nutritional importance. From the standpoint of total production, it occupies second place among the food plants of the world. It is the chief grain used in feeding livestock. It is also the chief article of diet of peoples in certain sections of the world, and in such sections the human nutritional disease, pellagra, is quite prevalent.

In recent years the above facts have been recognized, and it is now agreed that some emphasis in corn production should be placed on nutritive value as well as on the previously stressed characters which contribute to a high yield of sound corn on standing stalks. Any information concerning factors that may influence the value of corn as a source of the nutrients, protein, oil, and especially vitamins, would be of inestimable value.

Burkholder, McVeigh, and Moyer (1944) studied the niacin content of 94 samples of yellow corn and found a variation of 11.3 to 36.3  $\mu$ g of niacin per gram. The locality in which the corn was grown did not appear to have any influence on its niacin content, but a genetic factor may be responsible for the ability of the corn to store niacin. Teply, Strong, and Elvehjem (1942a) noted that environment influenced the niacin, pantothenic acid, and pyridoxine content of wheat. In another study (1942) they reported that the niacin content of corn varied from 1.56 to 2.60 mg %. Ellis and Madsen (1943), in summarizing the available information on the vitamin content of yellow corn, reported that the niacin content of 27 samples was 6.4 mg per pound, with a

<sup>1</sup> Published with the approval of the Director of the Ohio Agricultural Experiment Station.

range of 3.2 to 9.5 mg. The pantothenic acid content of nine samples of yellow corn varied from 2.72 to 4.54 mg per pound, with an average of 3.36 mg. Knox, Heller, and Sieglinger (1944) reported that sorghum grains varied little in riboflavin and pantothenic acid content, while niacin varied as much as 100%, depending on the place where the sorghum was grown.

It is evident that factors are involved which cause these variations in the niacin and pantothenic acid content of grains. These factors may be environmental, such as rainfall, soil, and length of growing season, and/or hereditary. The data reported in this paper show the niacin and pantothenic acid content of various corn hybrids.

### Materials and Methods

Nine well-known, double-cross hybrids were selected for analysis for the two vitamins. The hybrids were grown at five Middle West experiment stations over a period of three crop years.

An attempt was made to learn whether variations in vitamin content occurred among the hybrids and, if so, whether such variations could be attributed to hybrid, season, location, or the interaction of any two of these factors.

Preparation of the corn for analysis was as follows: Each sample was finely ground in a Wiley Mill and stored in a tightly closed glass jar in a dark room until analyzed. For niacin assays, an extract was prepared by adding 25 ml 1 *N* sodium hydroxide solution to 1 g of ground corn, autoclaving for 15 minutes at 15 pounds pressure, adjusting the pH to 6.6 to 6.8 with 1 *N* hydrochloric acid solution, and finally diluting the whole to 200 ml. For pantothenic acid assays, the extract was prepared by suspending 1 g ground corn in 75 ml distilled water, autoclaving for 15 minutes at 15 pounds pressure, diluting to 200 ml, and finally filtering with the aid of a thin layer of Celite 585 (Teply *et al.*, 1942a).

Niacin and pantothenic acid were determined microbiologically, using *Lactobacillus arabinosus* as the test organism. The basal medium was that of Krehl, Strong, and Elvehjem (1943). The medium, containing all the ingredients except the vitamins, was prepared at a concentration double that given by Krehl *et al.*, and was kept in the refrigerator under toluene until used. Immediately before each determination the vitamins (also kept in the refrigerator under toluene) were added to the required amount of the concentrated medium, the pH adjusted to 6.6 to 6.8, and the volume diluted to the recommended concentration of Krehl, *et al.*

The test organism was carried on liver tryptone agar stabs. For the inoculum, a 24-hour culture grown in basal medium with added

vitamin and distilled water was treated according to the method of Landy and Dicken (1942).

Triplicate tubes containing 0.05, 0.10, 0.15, 0.20, 0.40, 0.60, and 1.00  $\mu\text{g}$  niacin were used for the standard curve. For pantothenic acid, the amounts used were 0.01, 0.02, 0.04, 0.08, 0.12, 0.16, and 0.32  $\mu\text{g}$ . Duplicate tubes at three levels were used for each corn sample. Aliquots of the extract were of such size that the titrations at each of the three levels fell on the part of the curve which was most nearly linear. Incubation at 31°C for 72 hours was followed by titration with 0.1 *N* NaOH, using brom thymol blue as the indicator. With each determination a reference sample was assayed. The determina-

TABLE I  
NIACIN AND PANTOTHENIC ACID CONTENT OF DOUBLE-CROSS  
HYBRID CORN; 10% MOISTURE  
(MILLIGRAMS PER 100 GRAMS)

	1943		1944		1945	
	Niacin	Panto- thenic acid	Niacin	Panto- thenic acid	Niacin	Panto- thenic acid
Hybrid: Ohio C38						
Wooster, Ohio	2.15	0.54	2.08	0.36	1.92	0.47
Lafayette, Ind.	1.91	.51	2.43	.49	2.28	.64
Urbana, Ill.	2.20	.60			1.95	.52
Ames, Iowa	1.98	.69	2.28	.56	1.91	.60
Lincoln, Neb.	2.06	.61	2.50	.43		
Hybrid: Illinois 201						
Wooster, Ohio	2.00	.55	1.85	.36	1.85	.55
Lafayette, Ind.	2.06	.67	2.04	.38	2.35	.61
Urbana, Ill.	2.15	.56	2.01	.40	2.10	.95
Ames, Iowa	1.85	.72	2.27	.32	2.20	.64
Lincoln, Neb.	1.89	.78	1.65	.35		
Hybrid: Illinois 784						
Wooster, Ohio	1.63	.51	1.63	.31	1.43	.73
Lafayette, Ind.	1.87	.40	1.60	.29	1.80	.75
Urbana, Ill.	2.04	.44	1.65	.38	1.50	.61
Ames, Iowa	1.74	.48	1.83	.45	1.62	.79
Lincoln, Neb.	1.72	.64	1.58	.40		
Hybrid: Iowa 939						
Wooster, Ohio	2.46	.64	2.39	.45	2.59	.88
Lafayette, Ind.	2.27	.70	2.65	.48	2.95	.86
Urbana, Ill.	2.31	.70	2.72	.60	2.36	.67
Ames, Iowa	2.32	.51	2.77	.47	2.68	.78
Lincoln, Neb.	2.44	.81	2.58	.37		
Hybrid: U. S. 13						
Wooster, Ohio	1.87	.50	1.98	.21	1.78	.50
Lafayette, Ind.	2.06	.46	1.94	.35	1.94	.54
Urbana, Ill.	2.37	.77	2.32	.39	1.65	.42
Ames, Iowa	2.00	.51	2.23	.48	1.88	.64
Lincoln, Neb.	1.69	.74	1.72	.33		

TABLE I—*Continued*

	1943		1944		1945	
	Niacin	Panto- thenic acid	Niacin	Panto- thenic acid	Niacin	Panto- thenic acid
Hybrid: U. S. 35						
Wooster, Ohio	2.22	0.62 <sup>1</sup>	1.90	0.50	2.22	0.73
Lafayette, Ind.	2.27	.53	2.03	.48	2.45	.54
Urbana, Ill.	2.22	.47	2.18	.49	2.12	.44 <sup>1</sup>
Ames, Iowa	2.18	.55	1.88	.45	2.05	.60
Lincoln, Neb.	1.99	.68	1.86	.38	—	—
Hybrid: U. S. 44						
Wooster, Ohio	2.07	.50	1.92	.31	2.15	.53
Lafayette, Ind.	2.11	.42	2.36	.36	2.40	.48
Urbana, Ill.	2.24	.52	2.05	.39	2.17	.50
Ames, Iowa	2.14	.50	2.23	.54	2.25	.58
Lincoln, Neb.	2.00	.67	1.77	.35	—	—
Hybrid: Indiana 608C						
Wooster, Ohio	2.21	.43	2.25	.36	2.30	.53
Lafayette, Ind.	2.06	.42	2.59	.39	2.49	.56
Urbana, Ill.	2.23	.50	2.40	.51	2.31	.57
Ames, Iowa	2.17	.49	2.39	.60	2.49	.69
Lincoln, Neb.	2.04	.58	2.19	.47	—	—
Hybrid: Indiana 844D						
Wooster, Ohio	2.14	.44	1.92	.47	2.12	.56
Lafayette, Ind.	2.25	.40	2.44	.40	2.46	.39
Urbana, Ill.	2.30	.58	2.55	.44	2.32	.45
Ames, Iowa	2.19	.41	2.52	.64	2.63	.57
Lincoln, Neb.	2.18	.83	1.97	.50	—	—

tion was accepted if the reference sample gave a value within 10% of its known niacin or pantothenic acid content.

### Results and Discussion

The identity of the corn samples and the niacin and pantothenic acid content are shown in Table I. Variations in the content of both vitamins were found in all hybrids, for all three years and at all locations. For the whole study, the niacin values ranged from 1.43 to 2.95 mg per 100 g, with a mean value of 2.13; the pantothenic acid values ranged from 0.21 to 0.95 mg per 100 g, with a mean value of 0.53.

The mean niacin values for the hybrids, locations, and years are shown in Table II. The corresponding data for pantothenic acid are shown in Table III.

The data shown in Table I were subjected to analysis of variance and the results are shown in Table IV. Highly significant differences ascribable to hybrid, location, and year were found for both vitamins.

TABLE II  
MEAN NIACIN VALUES OF CORN HYBRIDS<sup>1</sup>  
(MILLIGRAMS PER 100 GRAMS)

	Ohio C38	Ill. 201	Ill. 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Ind. 608C	Ind. 844D
Wooster, Ohio	2.05	1.90	1.56	2.48	1.88	2.11	2.05	2.25	2.06
Lafayette, Ind.	2.21	2.15	1.76	2.62	1.98	2.28	2.29	2.38	2.38
Urbana, Ill.	2.08	2.09	1.73	2.46	2.11	2.17	2.15	2.31	2.39
Ames, Iowa	2.06	2.11	1.73	2.59	2.04	2.04	2.21	2.35	2.45
Lincoln, Neb.	2.28	1.77	1.65	2.51	1.71	1.93	1.89	2.12	2.08
1943	2.06	1.99	1.80	2.36	2.00	2.18	2.11	2.14	2.21
1944	2.32	1.96	1.66	2.62	2.04	1.97	2.07	2.36	2.28
1945	2.02	2.13	1.59	2.65	1.81	2.21	2.24	2.40	2.38
Three-year means	2.13	2.02	1.69	2.54	1.95	2.11	2.13	2.29	2.29

	Wooster, Ohio	Lafayette, Ind.	Urbana, Ill.	Ames, Iowa	Lincoln, Neb.	Year mean all locations
1943	2.08	2.09	2.23	2.06	2.00	2.09
1944	1.99	2.23	2.23	2.27	1.97	2.14
1945	2.04	2.35	2.05	2.19	—	2.16
Three-year means	2.04	2.22	2.17	2.17	1.99	2.13

<sup>1</sup> Values are expressed on a 10% moisture basis.

TABLE III  
MEAN PANTOTHENIC ACID VALUES FOR CORN HYBRIDS<sup>1</sup>  
(MILLIGRAMS PER 100 GRAMS)

	Ohio C38	Ill. 201	Ill. 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Ind. 608C	Ind. 844D
Wooster, Ohio	0.46	0.49	0.52	0.66	0.40	0.62	0.45	0.44	0.49
Lafayette, Ind.	.55	.55	.48	.68	.45	.52	.42	.46	.40
Urbana, Ill.	.56	.64	.48	.66	.53	.47	.47	.53	.49
Ames, Iowa	.62	.56	.57	.59	.54	.53	.54	.59	.54
Lincoln, Neb.	.52	.56	.52	.59	.54	.53	.51	.53	.67
1943	.59	.66	.49	.67	.60	.57	.52	.48	.53
1944	.46	.36	.37	.47	.35	.46	.39	.37	.49
1945	.56	.69	.72	.80	.53	.58	.52	.59	.49
Three-year means	.54	.56	.51	.64	.49	.53	.48	.51	.51

LOCATION MEANS

	Wooster, Ohio	Lafayette, Ind.	Urbana, Ill.	Ames, Iowa	Lincoln, Neb.	Year mean all locations
1943	0.53	0.50	0.57	0.54	0.70	0.57
1944	.37	.40	.45	.50	.40	.42
1945	.61	.60	.57	.65	—	.61
Three-year means	.50	.50	.53	.57	.55	.53

<sup>1</sup> Values are expressed on a 10% moisture basis.



TABLE IV  
VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS

Source of variance	Degrees of freedom	Niacin	Pantothenic acid
Location	4	0.2276**	0.0220**
Hybrid	8	.7907**	.0325**
Year	2	.0435 *	.3950**
Location × hybrid	32	.0217**	.0084
Location × year	8	.0769**	.0341**
Hybrid × year	16	.0851**	.0242**
Error	54	.0089	.0052
Total	124		

\* Significant.

\*\* Highly significant.

All three simple interactions indicated highly significant differences in niacin and pantothenic acid, with the exception of the interaction location × hybrid in the latter vitamin.

Tables V, VI, and VII, respectively, show the analysis of variance of the data for separate hybrids, years, and locations.

TABLE V  
VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF  
CORN HYBRIDS—INDIVIDUAL HYBRIDS

Source of variance	NIACIN								
	Ohio C38	Illinois 201	Illinois 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Indiana 608C	Indiana 844D
Location	0.0259	0.0637*	0.0186	0.0146	0.0600	0.0388*	0.0592*	0.0254	0.0951*
Year	.1127	.0321	.0538	.1197	.0523	.0799**	.0363	.0914**	.0323
Error	.0251	.0136	.0182	.0338	.0446	.0080	.0080	.0085	.0225

PANTOTHENIC ACID									
Location	0.0101	0.0085	0.0046	0.0049	0.0110	0.0087	0.0067	0.0113	0.0228
Year	.0196*	.1536**	.1407**	.1110*	.0781*	.0207	.0281	.0185*	.0026
Error	.0031	.0151	.0073	.0147	.0151	.0065	.0063	.0033	.0162

\* Significant.

\*\* Highly significant.

TABLE VI  
VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF  
CORN HYBRIDS GROWN FOR THREE YEARS

Source of variance	Niacin			Pantothenic acid		
	1943	1944	1945	1943	1944	1945
Location	0.0627**	0.1803**	0.1845**	0.0563**	0.0239**	0.0012
Hybrid	.1260**	.4243**	.4106**	.0224**	.0153**	.0431**
Error	.0117	.0268	.0111	.0064	.0039	.0099

\* Significant.

\*\* Highly significant.

TABLE VII  
VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF  
CORN HYBRIDS GROWN AT FIVE LOCATIONS

Source of variance	NIACIN				
	Ohio	Indiana	Illinois	Iowa	Nebraska
Hybrid	0.1944**	0.1867 <sup>1</sup> *	0.1369**	0.1997* <sup>2</sup>	0.1595 <sup>1</sup> *
Year	.0192	.1422**	.0937*	.0949 <sup>2</sup>	.0020
Error	.0128	.0336	.0314	.0250	.0265

PANTOTHENIC ACID					
Hybrid	0.0211 *	0.0223 *	0.0150	0.0025	0.0049
Year	.1321**	.0851**	.0402	.0572 *	.4252**
Error	.0057	.0084	.0155	.0118	.0062

\* Significant.

\*\* Highly significant.

*Variations Due to Hybrid.* The analysis of variance for the study as a whole (Table IV) showed a greater variation in niacin ascribable to hybrid than to any other factor.

The niacin content of Iowa 939 was the highest (mean 2.54 mg per 100 g) for all three years and at all locations. Illinois 784 assayed the lowest (1.69 mg per 100 g) in niacin for all three years and at all locations (Table II). Highly significant differences were observed within each year (Table VI) and at each location (Table VII). These hybrid differences indicate that the ability of the plant to elaborate niacin is inherited, but may be somewhat modified by environmental factors. However, at one location, Ohio (Table V), the hybrid differences were more highly significant than at any other location; likewise the hybrid differences during 1945 were more highly significant than for either 1943 or 1944.

The greatest variation in pantothenic acid was not ascribable to hybrid (Table IV), although a highly significant difference was due to this factor.

Iowa 939 also assayed highest (0.64 mg per 100 g) in pantothenic acid during each year and at each location. Hybrids assaying low in pantothenic acid were U.S. 13 (0.49 mg) and U.S. 44 (0.48 mg) (Table III).

In the analysis of variance of the data for separate years (Table VI), hybrid caused a highly significant difference in pantothenic acid within each year. Hybrid differences for pantothenic acid were significant in Ohio and Indiana but not in the other states (Table VII).

*Variations Due to Year.* The variation in niacin due to year (season) was significant for the whole experiment (Table IV). This

factor caused highly significant differences in niacin in U.S. 35 and Indiana 608C (Table V). This factor also caused a highly significant difference in Indiana and a significant difference in Iowa (Table VII).

The greatest variation found in pantothenic acid was ascribable to year (Table IV). The year 1944 produced corn having much less pantothenic acid (0.42 mg per 100 g) than that grown in 1943 (0.57 mg) and 1945 (0.61 mg) (Table III).

The analysis of data for separate hybrids (Table V) showed a highly significant difference in pantothenic acid due to year for Illinois 201 and Illinois 784; significant differences for Ohio C38, Iowa 939, U.S. 13, and Indiana 608C. Year (season) caused highly significant differences in pantothenic acid in Ohio, Indiana, and Nebraska, and a significant difference in Iowa (Table VII). This indicates that the inherited ability of the plant to elaborate pantothenic acid was greatly influenced by environmental factors.

*Variations Due to Location.* In the analysis of the entire data highly significant differences in both niacin and pantothenic acid were ascribable to location (Table IV), although, as was stated earlier, the greatest variation in niacin was due to hybrid and the greatest variation in pantothenic acid was due to year (season). Location had a greater influence on niacin than on pantothenic acid. Corn grown at the Indiana location was highest in niacin (2.22 mg per 100 g) and that grown at the Nebraska location was lowest (1.99 mg) (Table II). Corn grown at the Iowa location was highest in pantothenic acid (0.57 mg), while that grown at the Indiana and Ohio locations was lowest in pantothenic acid (0.50 mg) (Table III).

In the analysis of the data for separate hybrids (Table V) significant differences in niacin ascribable to location were found only for Illinois 201, U.S. 35, U.S. 44, and Indiana 844D; the differences in pantothenic acid due to location were not significant.

The analysis of the data for separate years (Table VI) showed that location caused highly significant differences in niacin during each of the three years and in pantothenic acid during 1943 and 1944.

### Summary

Samples of nine double-cross corn hybrids grown at five experiment stations for two years, and the same hybrids grown for an additional year at four of the five stations, were assayed for niacin and pantothenic acid. Variations for both vitamins were found in all hybrids for all three years and at all locations.

The niacin content of Iowa 939 averaged the highest for all years (2.54 mg per 100 g) and locations, while Illinois 784 assayed the lowest (1.69 mg per 100 g) in niacin.

The niacin values for all samples ranged from 1.43 to 2.95 mg per 100 g, with a mean value of 2.13 mg.

Iowa 939 had the highest pantothenic acid content (mean 0.64 mg per 100 g), and U.S. 44 and U.S. 13 were low in pantothenic acid.

The pantothenic acid values for all samples ranged from 0.21 to 0.95 mg per 100 g, with a mean value of 0.53 mg.

In the analysis of variance for niacin, the greatest variation was ascribable to hybrid.

The greatest variation in pantothenic acid was ascribable to year (season).

• The influence of hybrid and year factors on niacin and pantothenic acid, respectively, indicated that the inherited ability of the plant to elaborate niacin was less subject to the influence of environmental factors than was the inherited ability of the plant to elaborate pantothenic acid.

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## RELATIONSHIP BETWEEN MOISTURE CONTENT OF FLOUR AND HUMIDITY OF AIR

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(Presented at the annual meeting, May 1947; received for publication May 23, 1947)

Early workers observed that there is a close relationship between the physical properties of flour and the humidity of the atmosphere, and in recent times several authors have reported on the relationship existing between the moisture content of flour and the humidity of the atmosphere surrounding the flour at controlled temperatures. Bailey (1920) and Anker, Geddes, and Bailey (1942) have presented a comprehensive review of the moisture content of wheat flour at various relative humidities. Fairbrother (1929) investigated the weight of flour as related to relative humidity.

No information on the relationship between the moisture content of flour and the atmospheric humidity at temperatures appreciably higher than room temperature has been found in the literature. The object of the present investigation was to supply information on the flour moisture content-humidity-temperature relationship over an extended range. A simple method has been employed to determine the variation of flour moisture content over the range of 50° to 95°C at humidities ranging from approximately zero to 80% relative humidity.

### Materials and Methods

A typical southwestern wheat flour analyzing 11.5% protein and 0.45% ash (14% moisture basis) was used in this study. An amylograph test made according to the procedure of Selman and Sumner (1947) gave a malt index of 500; hence the flour was adequately diastated.

TABLE I  
RELATIVE HUMIDITY OF AIR IN EQUILIBRIUM WITH SULFURIC  
ACID SOLUTIONS AT 50°, 75°, AND 95°C

Solution	Percentage H <sub>2</sub> SO <sub>4</sub> (by weight)	Relative humidity of equilibrium air		
		50°C	75°C	95°C
A	26.12	80.1	81.6	82.1
B	40.08	58.1	59.1	59.8
C	52.27	33.1	35.0	37.3
D	83.58	0.44	0.68	0.97

For each temperature and relative humidity 2 g of flour were weighed in quadruplicate into a glass-stoppered weighing bottle, and,

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with the stopper removed, placed in a laboratory desiccator containing 170 ml of a sulfuric acid solution which would give the desired relative humidity. The desiccator was then stored in a laboratory oven at the desired temperature, and the weight of each sample was checked periodically until the weight became constant.

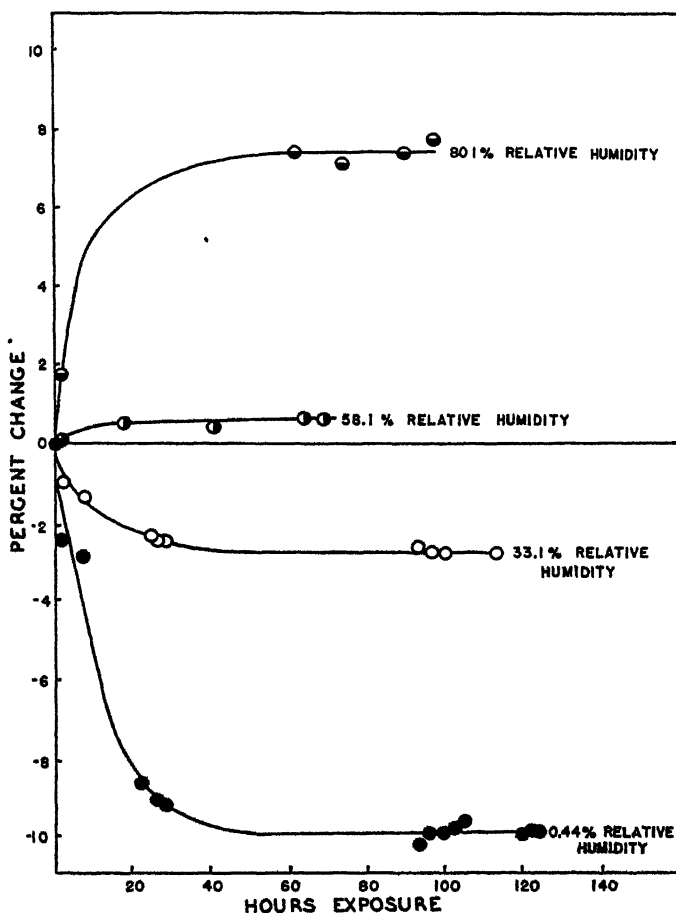


Fig. 1. Moisture variation of flour at 11.36% moisture content upon exposure at 50°C to air of different relative humidities.

The sulfuric acid solutions used were made from reagent grade concentrated sulfuric acid, the concentrations of which were determined by means of both hydrometers and titration against standard sodium carbonate solutions. The relative humidity of air in equilibrium with each of the solutions was calculated from the data of Wilson (1921)

and from the International Critical Tables (1928). The calculations are recorded in Table I.

At the completion of the experiment the specific gravity of the sulfuric acid solution was checked. In every experiment, there was less than 1% change in the specific gravity.

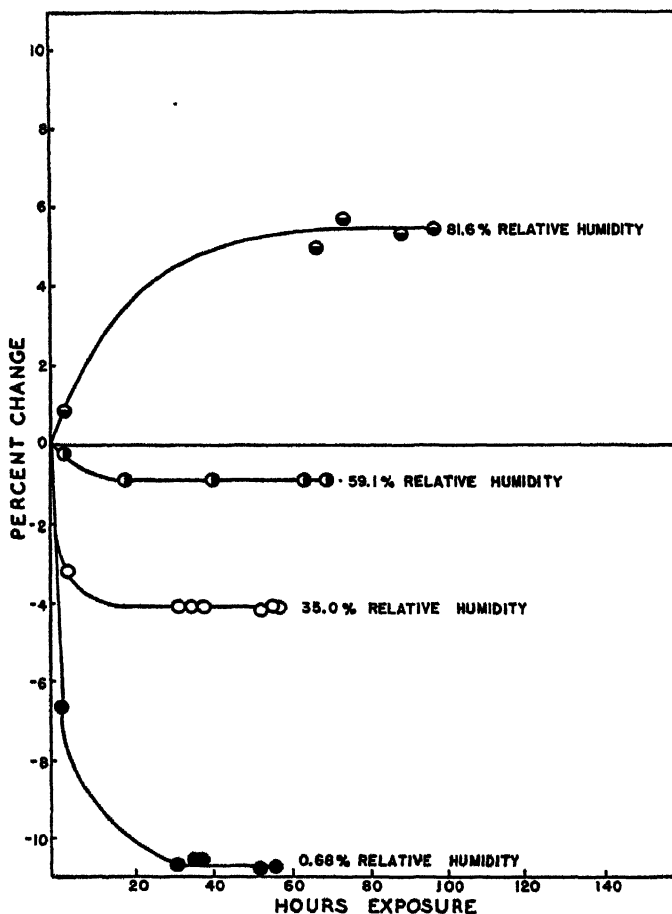


Fig. 2. Moisture variation of flour at 11.36% moisture content on exposure to air at 75°C.

### Results and Discussion

The percentage change in the moisture content of the flour was plotted as related to time in the oven. Figures 1, 2, and 3 show the results. The values are based on the average of quadruplicate determinations.

The original moisture content of the flour was determined by the air-oven method and was found to average 11.36%. The data of Figure 1 show that about 50 hours are required for all samples to reach equilibrium at 50°C and 75°C as compared with about 40 hours of exposure for the flour at 95°C. However, as the temperature increased

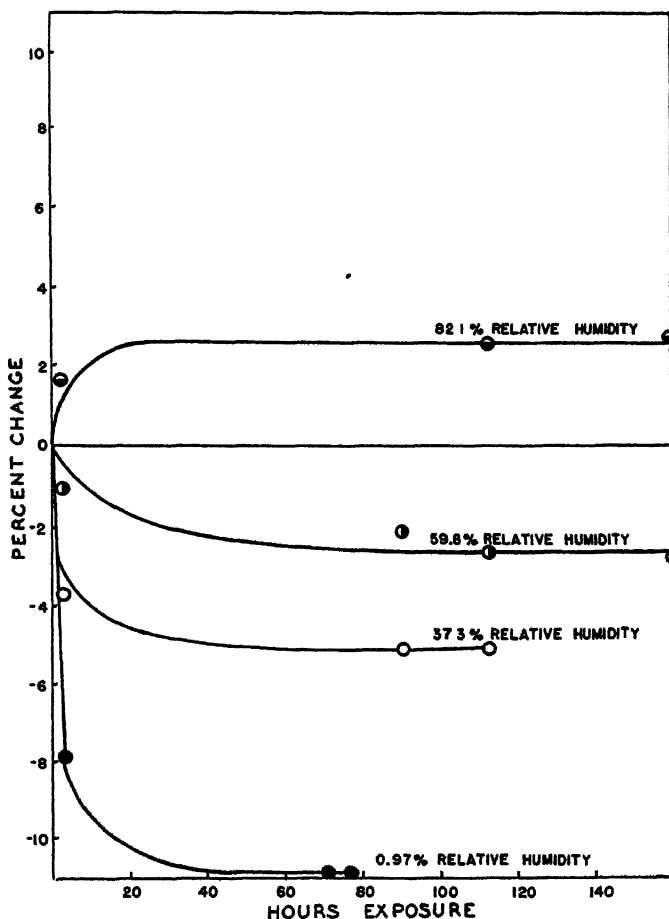


Fig. 3. Moisture variation of flour at 11.36% moisture content on exposure to air at 95°C.

the time required for reaching equilibrium at any given humidity was diminished somewhat.

The relationship existing between the relative humidity of air and the moisture content of the flour is represented graphically in Figure 4, and numerical data for selected relative humidities are shown in Table II.



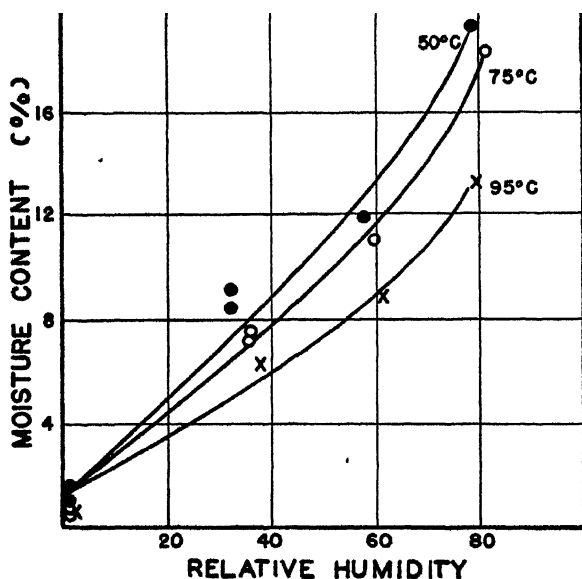


Fig. 4. Moisture content of flour at 50°C, 75°C, and 95°C in equilibrium with atmospheres of different relative humidities.

The moisture content of flour in equilibrium with air of low relative humidity varies little with the temperature. With increasing relative humidities the moisture contents increased at a rate slightly more than linearity at all temperatures within the range studied.

Although it is customary to express the moisture content of air in terms of the degree of saturation of the air, i.e., relative humidity, a

TABLE II

MOISTURE CONTENT OF FLOUR IN EQUILIBRIUM WITH AIR OF VARIOUS RELATIVE HUMIDITIES AT DIFFERENT TEMPERATURES

Air temperature = 50°C			Air temperature = 75°C			Air temperature = 95°C		
Air moisture		Flour moisture	Air moisture		Flour moisture	Air moisture		Flour moisture
R. H.	Water vapor pressure		R. H.	Water vapor pressure		R. H.	Water vapor pressure	
%	mm Hg.	%	%	mm Hg.	%	%	mm Hg.	%
80.1	75.5	18.75	81.6	236	16.58	82.1	520	13.96
58.1	53.7	10.98	59.1	171	10.57	59.8	379	8.63
33.1	30.6	8.19	35.0	100	6.85	37.3	237	6.24
		8.56			7.20			
0.44	4.06	0.91	0.68	19.6	0.36	0.97	61.5	0.48
		1.41			0.64			

more absolute definition of the value is the water vapor pressure in millimeters of mercury. Figure 5 demonstrates that over the range studied the moisture content of the flour is a linear function of the water vapor pressure for a given temperature.

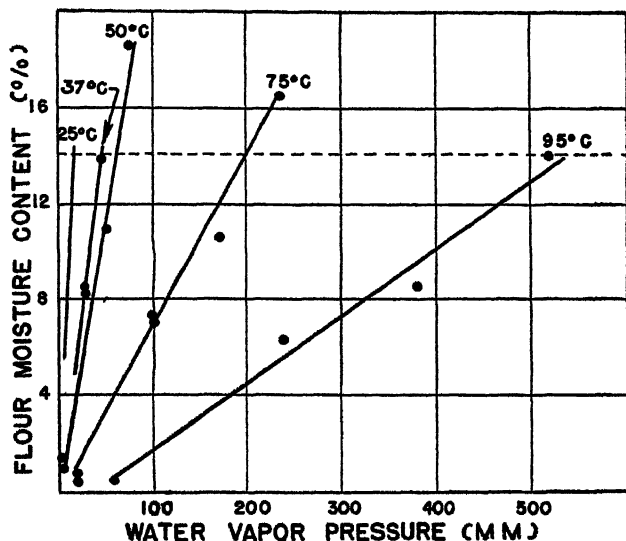


Fig. 5 Isothermal relationships of flour moisture content to water vapor pressure

In addition to the experimental results presented in this paper the moisture content of flour in equilibrium with air of various humidities at 25° and 37°C has been calculated from the data of Anker, Geddes, and Bailey (1942), and is included in Figure 5. The isothermal relationships between the flour moisture content and the water vapor pressure have been computed by the method of least squares and the regression coefficients and ordinate intercepts are listed below.

Temperature, degrees C	Regression coefficient	Ordinate intercept
25	0.585	4.08
37	0.302	0.249
50	0.233	0.433
75	0.0720	-0.0721
90	0.0283	-1.13

Since commercial flour generally has a moisture content in the neighborhood of 14%, it is interesting to note the water vapor pressure giving this value at all of the temperatures listed in the above table. As shown in Figure 6, the relation between temperature and the logarithm of the water vapor pressure is essentially linear, the regression

equation being:

$$t_{14\%} = 48.6 \log_{10} (V.P.)_{14\%} - 37.4$$

when  $t_{14\%}$  and  $V.P._{14\%}$  are the temperature and water vapor pressure, respectively, required to give flour of 14% moisture content.

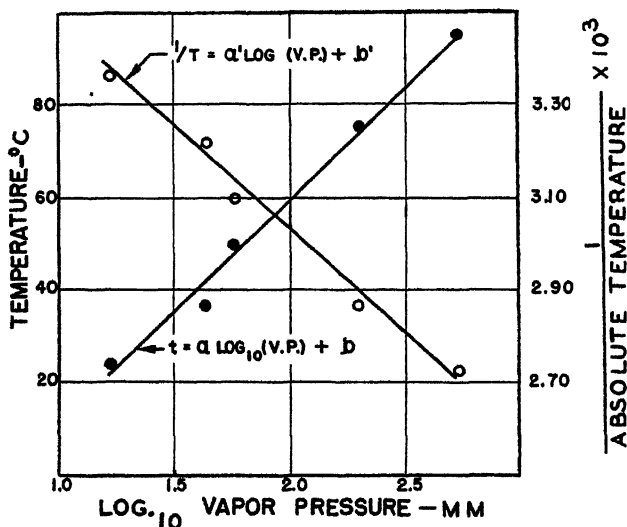


Fig. 6. Relationship of temperature to the water vapor pressure of flour at 14% moisture content.

This evaluation of the data could be extended to any moisture content by interpolation of the graphs to any desired value and relating the temperature to water vapor pressure.

Although practical requirements make it more desirable to express temperature in the units of the Centigrade scale, theoretical considerations reveal that the reciprocal of the absolute temperature is preferable. From the Clausius-Clapeyron equation, the logarithm of the vapor pressure of a liquid is approximately a linear function of the reciprocal of the absolute temperature and the slope of the line expressing this relation can be used to calculate the heat of vaporization. This convenient relationship has been used by Neale and Stringfellow (1941) to determine the heat of sorption of cotton from data similar to those presented in this paper.

Reconsideration of the values shown in Table II show that for the flour at 14% moisture content the relationship is:

$$\frac{1}{t_{14\%}} = 0.00392 - 0.000443 \log_{10} (V.P.)_{14\%}$$

when  $T$  is the absolute temperature and V.P. is the water vapor pressure. The equation is graphically represented in Figure 6. From the regression coefficient the heat of dehydration of the flour is calculated as 10.7 Calories per mole of water. Winkler and Geddes (1931) arrived at a value of 2.96 Calories per mole of water as the energy of adsorption at a flour moisture content of 14% in the presence of an excess of liquid water. The value of 10.7 Calories per mole is in reasonable agreement if the heat of vaporization is taken into consideration.

### Summary

The equilibrium moisture contents of a typical Kansas flour in contact with air of varying moisture content at 50°, 75°, and 95°C were determined. A linear relationship exists between flour moisture content and the absolute humidity of the air. For a moisture content of 14%, the reciprocal of the absolute temperature is a linear function of the logarithm of the water vapor pressure. The heat of dehydration has been calculated as 10.7 Calories per mole of water.

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# IMPROVED FLOUR BLEACHING PROCESS USING CHLORINE DIOXIDE PRODUCED BY AN ELECTRIC ARC METHOD <sup>1</sup>

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(Presented at the Annual Meeting, May 1947; received for publication June 24, 1947)

Nitrogen oxides have been used for the bleaching of flour for many years. Several methods of obtaining these nitrogen oxides at low partial pressures have been proposed and tried commercially; but the passage of air through a continuous electric arc seems to have proved the most successful for flour bleaching purposes. Alsop machines in this country and Brabender equipment in Europe employing the electric arc method have been in wide use for a long time. Many of these generators are in use at the present time; but others are idle in mills or warehouses. The reasons for the decreased usage of the process in this country in the past decade or so are twofold. First, as shown by Ferrari, Hutchinson, and Mecham (1945) the color removal with nitrogen oxides is not nearly as great as that which can be achieved with nitrogen trichloride and benzoyl peroxide. Second, the desirable effects on the baking properties of certain types of flours that are achieved with nitrogen trichloride and chlorine dioxide cannot be brought about by the application of nitrogen oxides from the electric arc machines to flours.

The purpose of this paper is to show how the electric arc generators for producing nitrogen oxides can be converted to bleaching generators capable of producing chlorine dioxide or a mixture of chlorine dioxide and nitrogen oxides diluted with air. A greatly improved bleaching process results. Flexibility in the proportions of the two reagents, a much greater degree of color removal, and desirable maturing effects on the flour resulting in improved baking properties are achieved, completely overcoming the above noted drawbacks to the nitrogen oxide bleaching process. The effects of chlorine dioxide on the baking properties of flour have been discussed by Ferrari, Hutchinson, Croze, and Mecham (1941). In most instances they are quite similar to those produced by nitrogen trichloride.

## Equipment and Procedure

Much of the equipment and many of the procedures used in these experiments were described in the recent paper by Hutchinson, Derby,

<sup>1</sup> Paper No. 81, Journal Series, General Mills, Inc., Research Laboratories.

and Ferrari (1947). A diagram of the equipment for the small-scale experiments is shown in Figure 1.

This process for producing chlorine dioxide is controlled by generating known amounts of nitrogen oxides (Hutchinson *et al.*, 1947) and contacting them with a large excess of technical grade flaked sodium chlorite (such as Mathieson Alkali Works, Inc., "C<sub>2</sub>") in a vertical column. For the small-scale experiments reported in this paper the effluent gases from the Alsop equipment were proportioned by flow-meters, the needed amount being conducted to the chlorite tower and the excess being exhausted to a hood or the outside atmosphere. In

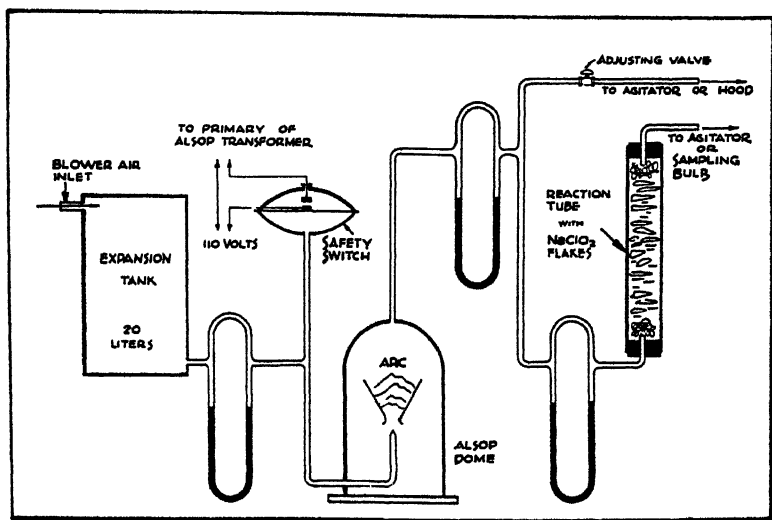


Fig. 1. Diagram of the pilot scale equipment used in the manufacture of ClO<sub>2</sub> gas with the Alsop generator.

commercial application there would be no need for wasting any of the Alsop gases. Continuous operation would be achieved by the use of a multiplicity of chlorite towers, the nitrogen oxides being introduced to some of them, while the others were being charged with fresh chlorite. Suitable multiple chlorite tower equipment has been described by Woodward, Petroe, and Vincent (1944).

The effluent gases from the chlorite towers, consisting of chlorine dioxide or a mixture of chlorine dioxide and nitrogen oxides, were conducted to MacLellan Batch Mixers for the flour bleaching process.

One of the outstanding advantages of the process is the apparent ease of safeguarding the generation of chlorine dioxide in this manner. The Alsop equipment is only capable of generating nitrogen oxides at low partial pressures of 3 mm Hg or less when operating at optimum

capacities. Moreover, the current to the electrodes forming the arc is shut off by means of a diaphragm contact activated by the air supply to the nozzle, when the air pressure to the nozzle beneath the arc falls below a predetermined level. Although the exact course of the reaction between the nitrogen oxides and the chlorite has not been determined, it appears that about one mole of chlorine dioxide is produced from each mole of nitrogen peroxide. Thus, the partial pressure of the chlorine dioxide is automatically kept at about 3 mm Hg or less, far below the safe partial pressure for chlorine dioxide which is about 30 mm Hg according to Woodward *et al.* (1944). The necessary equipment for safeguarding the process is therefore already available in the Alsop machine with the air pressure-operated contact switch.

### Experimental

Some samples of a patent flour milled from a spring wheat blend were experimentally bleached with Alsop gases. Others were treated with Alsop gases which were conducted through a 40-inch column 1 5/16 inches in diameter containing Mathieson Alkali Works, Inc., "C<sub>2</sub>" chlorite. The results shown in Table I indicate that the bleaching

TABLE I

BLEACHING OF PATENT FLOUR WITH NITROGEN PEROXIDE PRODUCED  
IN AN ALSOP MACHINE AND WITH ALSOP GASES CONVERTED TO  
CHLORINE DIOXIDE BY PASSAGE THROUGH A CHLORITE COLUMN

Bleaching treatment per cwt. of flour	Pekar or "slick" score	"Carotene" <sup>1</sup> p.p.m.	Bread crumb color
Unbleached flour	creamy	1.98	—
0.9 g nitrogen peroxide <sup>2</sup>	7 creamy	1.01	10 creamy
0.9 g nitrogen peroxide-repeat	7 creamy	1.05	—
0.9 g nitrogen peroxide through a column of chlorite <sup>3</sup>	10 white	0.77	12 white
Repeat of above treatment	10 white	0.77	—
1.0 g nitrogen peroxide through the chlorite column	10 white	0.69	—

<sup>1</sup> Flour color expressed as naphtha-alcohol "carotene"; determined as outlined in *Cereal Laboratory Methods* (4th ed. 1941).

<sup>2</sup> This sample was used as standard for the bread crumb color tests.

<sup>3</sup> This sample was used as standard for the Pekar tests.

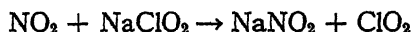
potency of the gases is very greatly increased by passage through the chlorite column. Both the slick and bread crumb colors were improved and whitened. The carotene contents of the treated flours clearly reflected the greater bleaching power due to the conversion of the nitrogen oxides to chlorine dioxide by the passage through the chlorite column. Carotene contents of 0.77 and 0.69 p.p.m. are in the range of commercially bleached flours.

In these preliminary experiments the conversion of the nitrogen oxides in the Alsop gases to chlorine dioxide was not a hundred percent complete; but it was believed to have been substantially so by the bleaching results and by the analyses of effluent gases from the Alsop equipment and from the chlorite tower. The odor of the effluent gases from the chlorite tower indicated the presence of some unreacted nitrogen oxides.

Woodward *et al.* (1944) described a method of analysis for chlorine dioxide gas which involves absorbing the gas in a 10% potassium iodide solution made from chlorine-free water, adding a starch indicator, and titrating to a colorless end point with 0.1 *N* sodium thiosulfate solution. The solution is then acidified with an excess of 30% acetic acid and is again titrated with the same strength sodium thiosulfate. One-fifth of the total sodium thiosulfate corresponding to the chlorine dioxide is used in the first titration and four-fifths is used in the second titration. Thus the ratio of the thiosulfate consumed in the first titration to that in the second is 1:4 for pure chlorine dioxide. Chlorine titrates in the first titration, so the ratio is rapidly increased, if appreciable quantities of chlorine are present.

Analysis of the effluent gases from an Alsop machine, consisting of nitrogen oxides and air predominantly, resulted in a ratio of approximately 1:10, far different from the 1:4 ratio secured with pure chlorine dioxide. Gases obtained by passing the effluent gases from an Alsop machine through a column of sodium chlorite were analyzed in the same manner and the ratio of the first to the second titration value was found to be approximately 1:4. This indicates that the nitrogen oxides were converted practically quantitatively to chlorine dioxide.

The conversion of the nitrogen oxides to chlorine dioxide in this process can probably be expressed by the following equation, although the exact course of the reaction is not known:



Optimum methods of utilizing chlorine dioxide and nitrogen peroxide in multiple flour bleaching combinations have been described by Hutchinson and Derby (1945). Their data indicate that maximum color removal is achieved by the sequential application of chlorine dioxide followed by nitrogen peroxide. Application in the reverse order or simultaneously results in appreciably less color removal.

Flexibility in bleaching treatments can be achieved by splitting the effluent gases from an Alsop or Brabender machine, conducting one portion through a chlorite column and then to the first flour agitator, and conducting the second portion with no conversion treatment directly to the second flour agitator.



### Summary

A process is described for converting the effluent nitrogen oxide gases from electric arc equipment such as the Alsop or Brabender machines to chlorine dioxide by passage through a bed or column of technical grade sodium chlorite. The converted gases exhibit much greater bleaching power and also possess the ability to alter the baking properties of certain types of flours which is characteristic of chlorine dioxide and which is similar in many instances to that possessed by nitrogen trichloride.

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## PROTEIN AS A FACTOR IN SOFT WHEAT MEAL MIXOGRAM AREA

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(Presented at the Annual Meeting, May 1947; received for publication May 21, 1947)

The mixogram offers possibilities as a means of evaluating and classifying new strains of wheat. In the early stages of a breeding program the number of lines is large and the grain from each very limited in amount. Because of the large number of lines, the time factor makes micro milling unfeasible, but a sifted meal may be used to make mixograms very similar to those made from flour. A method has been described by Lamb (1944) for preparing the meal from small amounts of wheat. Morris, Bode, and Heizer (1944) found that the area under the flour mixogram, measured with a planimeter, has a general relationship to quality of soft wheats and to the type of products for which they may be utilized. Sifted meal mixograms are equally useful for this purpose.

Given mixograms from two strains of wheat, the factor most likely to interfere with comparison of mixogram areas is protein content. Differences in protein, as Swanson (1940) and also other workers have pointed out, affect the height of curve, but have little or no influence on the time required to reach minimum mobility. Consequently the angles of the ascending and descending slopes are steeper in curves from higher protein samples. The area under the mixogram also increases. Measurements of mixogram area then are affected by protein level, and this complicates comparisons. The purpose of the present study was to evaluate this effect of protein on sifted wheat meal mixograms.

### Experimental

A series of 21 nursery lines was taken from the advanced nursery tests grown at four stations in Ohio for two years. These selections had been advanced from preliminary tests, and, so far as could be judged from appearance, were of satisfactory soft wheat quality. Mixograms and protein analyses were made for each of the 168 samples. Mixogram areas were measured for a six-minute interval.

Preliminary analysis of the data showed there were highly significant differences in mixogram area. Covariance analysis was then made. When the data were considered as eight station-year groups, thus eliminating variety as a factor, protein differences accounted for nearly all the variations in mixogram area. Figure 1 shows graphically that the protein content did account for differences in mixogram area. The range in meal protein was 9.9 to 12.3%, a range not greater than that encountered in commercial samples of Ohio wheat. The close arraignment to the regression line is not due to an artificially expanded series. Neither station nor season introduced significant variation in mixogram area. Harris, Sibbitt, and Elledge (1944) report analogous data from six spring wheats grown at four stations for two seasons. They found significant differences in curve height between seasons. This does not contradict the present findings, since curve height is closely associated with protein level.

Next, the data were grouped by variety. The covariance analysis showed that protein did not explain all differences in mixogram area between varieties; the area differences, independent of protein, were considerable, and were highly significant statistically. The data are presented graphically in Figure 2.

The data present some further points of interest. In Figure 2, the regression line for mixogram area on protein is indicated for each of the 21 varieties. There is variability in the slopes of these lines, which is not surprising since each regression is determined from only eight samples. Statistically there is no indication that the slopes of the

regression lines are not homogeneous. The average regression therefore should provide a satisfactory measure of the change in mixogram area to be anticipated with different protein levels. This average regression is shown in Figure 2, and indicates an increase of about 4.5 sq cm in mixogram area for each 1% increase in protein of the wheat meal. Adjusting mixogram areas to protein content reduced the mean square for error within varieties from 24.08 to 8.38, and furthermore the reduction in sum of squares due to regression was very large

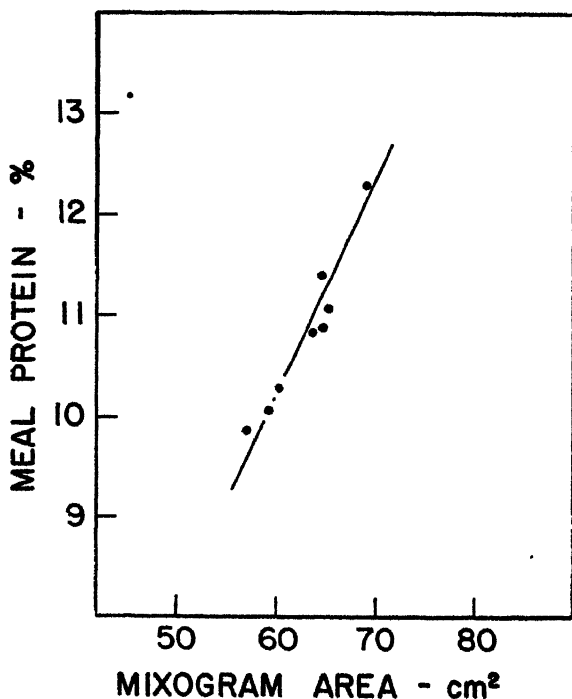


Fig. 1. Relation between meal protein and mixogram area in 8 tests, each including the same 21 varieties of soft red winter wheat.

and highly significant. In Figure 1, the regression from station means agrees very closely with this figure, indicating about 4.6 sq cm for each 1% protein in the wheat meal.

Morris in his work at the Federal Soft Wheat Laboratory (unpublished data) has found a greater increase in soft wheat flour mixogram area for each percent increase in protein, namely 7 sq cm. Most, but not all, of this difference would have been anticipated because Morris measures area under flour mixograms for a seven-minute time interval rather than the six minutes used for sifted meal mixograms,

and flour curves have greater areas even when the same time interval is used. A slightly higher absorption was used for the wheat meal mixograms, and this would also tend to give a smaller increase in area for each increment in protein.

Mixogram area does not give a complete picture of soft wheat quality; it does not even give all the information to be obtained from the mixogram. However, it is considered one of the best single tests

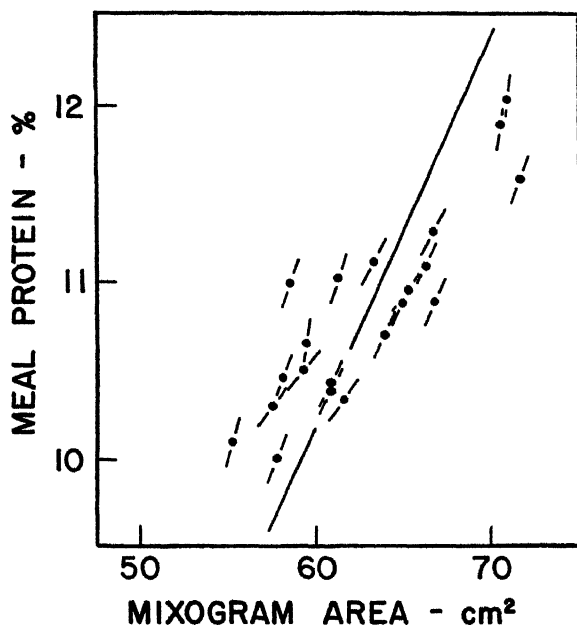


Fig. 2 Relation between meal protein and mixogram area for 21 varieties of soft red winter wheat, each grown in the same 8 tests. Regressions for each variety and average regression are also shown.

that have been developed for evaluating new selections at an early stage in the soft wheat breeding program at the Ohio Station.

### Summary

Area under the mixogram is influenced by both variety and protein content. In the case of soft wheats the influence of protein may be reduced to insignificant proportions by adjusting six-minute sifted meal mixogram areas 4.5 sq cm for each 1% protein. When the influence of protein has been largely eliminated in this way there remain highly significant differences in area which are due to variety, and which may be used as a measure of quality differences.

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## COMMUNICATION TO THE EDITOR

A Spot Test for the Identification of Rodent Urine  
on Packaged Commodities

Received June 8, 1947

SIR:

The use of the "black light" for the detection of rodent contamination on bagged commodities has presented some difficult problems in that many ingredients used in the milling and baking industry fluoresce under ultraviolet light. While each substance has its characteristic color, many are of a similar shade, often confusing the operator. Three of the common tests for urine are the urease test for urea, the xanthidrol test for urea, and the extraction of urea and crystallization of urea nitrate (*Methods of Analysis A.O.A.C.*). These tests all involve cutting out a portion of the stained cloth, chemical treatment, and observation of characteristic crystals under the microscope.

A simple spot test which may be used at the scene of investigation has been devised. Three grams of para-dimethylamino benzaldehyde are dissolved in 25 ml of ethyl alcohol and made up to 100 ml with a saturated solution of oxalic acid. By applying this solution to a suspected urine stain with a camel's-hair brush, a chrome yellow color will develop if urine is present. This test works well on cotton, paper, and burlap bags.

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## RELATIONSHIP OF THE PHYSICAL PROPERTIES OF WHEAT FLOUR TO GRANULATION<sup>1</sup>

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(Presented at the Annual Meeting, May 1947, received for publication July 14, 1947)

In a previous investigation of flour granulation it was shown that the wheat endosperm particles passing through a flour cloth during sieving do not always approximate the size of the aperture openings of that cloth (Shollenberger, 1921). Recent work indicates that the particles vary in size from 150  $\mu$  to approximately 5  $\mu$  in diameter. LeClerc, Wessling, Bailey, and Gordon (1919) investigated the chemical composition of different-sized flour particles and showed that flour sifted through a fine silk bolting cloth had inferior baking properties to that sifted through coarser bolting cloth. These workers also found that flour sifted through a coarser flour cloth was only slightly better in baking properties than that sifted through the finest cloth, while the intermediate flour was found to give the best loaf. This suggests that the coarsest and the finest particles of flour had a detrimental influence on the whole flour. Maun (1927) and Kress (1929) substantiated the work of LeClerc *et al.* (1919). Pulkki (1938) and Swanson (1938) found that the flour particles passing through a fine flour cloth contained less protein than the coarser flour particles.

The difficulty confronting the past investigators in making a complete fractionation of flour into well-defined particle size groups was probably due to the mesh fineness limitations of silk flour cloths, and to the tendency for flour particles to agglomerate. The finest mesh silk flour cloth (25 standard) does not have aperture openings of a well-defined size or shape. The average aperture size openings of the 25 standard cloth is 63  $\mu$ , or approximately twice the size necessary to make a more complete particle size fractionation of flour.

Previous work on the relation of flour granulation to the chemical characteristics of flour has been limited largely to the reporting of

<sup>1</sup> This paper represents a portion of a thesis presented to the Graduate School of Kansas State College in partial fulfillment of the requirements for the degree of Master of Science.

<sup>2</sup> Contribution No. 142, Department of Milling Industry.

trends. It was the purpose of this investigation to make a complete fractionation of flour into several well-defined particle size groups, and to determine the physical and chemical characteristics and baking qualities of each group.

### Materials and Methods

A commercially milled hard red winter, straight grade wheat flour was used throughout this investigation. The flour was fractionated

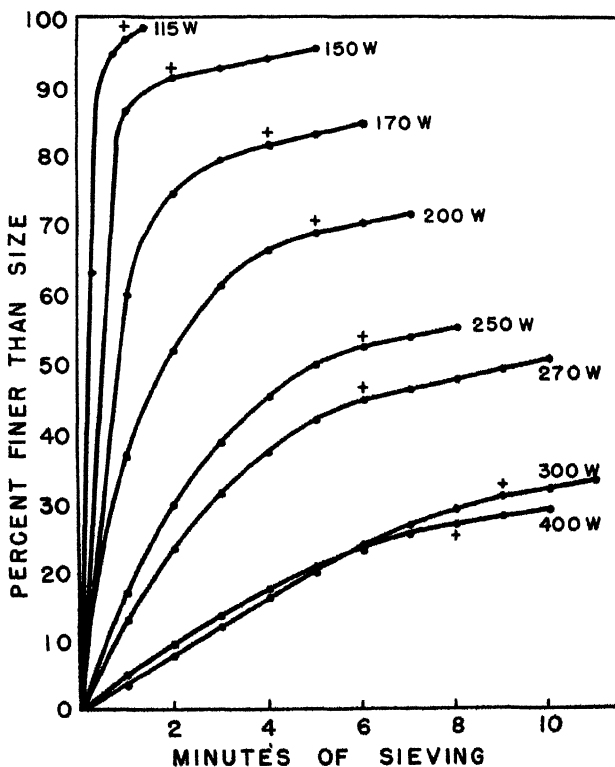


Fig 1. Points establishing the optimum percentage of material through each wire testing sieve.

into 12 different particle size groups using a Ro-Tap sieve shaker equipped with W. S. Tyler standard wire sieves nos. 115, 150, 170, 200, 250, 270, 300, and 400. Following the sieving separation the fractions were subjected to an air elutriation treatment employing the Roller Particle Size Analyzer.

*Separation by Sieves.* The particle size distribution in the flour was accomplished by using one testing sieve at a time in the Ro-Tap shaker. The sieve was stacked upon a coarse wire screen carrying the under-sieve brush cleaners, and these two screens were then

stacked upon the pan. A small cloth cleaner was used for the top side of the testing sieve. A 50-g. sample was introduced onto the testing sieve and the sieving operation repeated at one-minute intervals. After each minute of operation, the top sieve was carefully removed and weighed. Extreme care was taken when removing and replacing the sieve not to disturb the "lay" of the material. This procedure was continued and the results followed by plotting a curve (Figure 1) of the percentage of throughs of the sieve against each min-

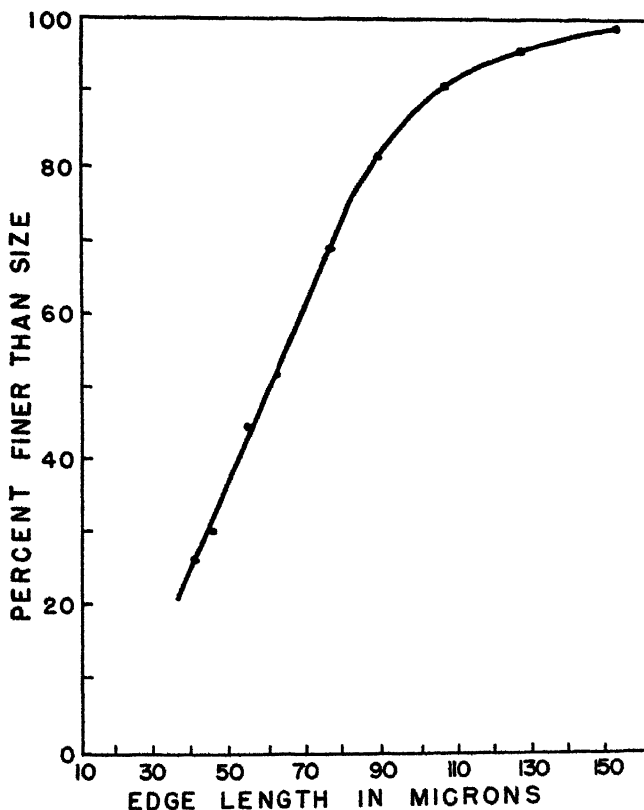


Fig 2 Flour particle size distribution curve

ute of sieving, until a point was reached in the curve after which essentially a straight line occurred. After this point was reached, additional material removed by continued sieving was probably the result of the reduction of particle size by attrition.

All remaining sieves were treated in the above described manner. The points, having been established when all of the sieving is completed, indicate the optimal percent of material through the sieve, and are illustrated by the granulation curve in Figure 2.



The sieving method just described was used to establish the flour granulation curve; but to obtain a larger quantity of the various particle sizes for thorough testing, a modified sieving procedure was employed.

A 50-g. sample of flour was sifted over the No. 400 mesh sieve for the number of minutes necessary to produce the optimal percentage of material through the sieve, as determined from the granulation curve. The overs of the sieve were then removed and another 50-g. sample of flour was sifted. The overs of the sieve were again removed and the procedure continually repeated until a large quantity of the sieve throughs was obtained. The next coarser mesh sieve, the No. 300, was used for sifting the material taken from the overs of the No. 400 mesh sieve, and this procedure was repeated for all of the succeeding coarser mesh sieves. Using the finest wire mesh sieve initially was necessary so that the coarser material would carry the finest particles to the mesh openings, permitting free bolting.

The particles passing through the No. 400 mesh sieve constituted the 0–38  $\mu$  fraction. The particles passing through the No. 300 mesh sieve made up the 38–46  $\mu$  fraction, and so on, until a complete fractionation of flour into its component particle size groups was accomplished.

*Separation by Air.* Flour particles have a tendency to agglomerate, and the agglomerates are not broken up entirely during the sieving process. Thus the accuracy of the particle size separation by sieving is limited. Also, sieving does not remove extremely fine or pulverized bran chips, dirt, or foreign material. However, the breaking of the flour agglomerates by air elutriation is quite effective. By this method it is possible to remove completely bran chips and all other contaminating material. The air separation principle was used on the particle size fractions obtained from sieving in this study.

The Roller Particle Size Analyzer (Figure 3) employs the air elutriation principle. The determination of removal of a particle size of powdered material below the 110- $\mu$  range is accomplished by means of a carefully regulated current of air. Any number of particle size fractions may be obtained. The air required for separation is regulated in accordance with Stokes' Law for falling bodies.

The analyzer, shown in Figure 3, consists of an air jet inlet (A), U-shaped glass vessel for holding the flour sample (B), oscillation connections for the latter, a series of four stainless steel settling chambers (9, 4½, 2¼, and 1⅛ inches in diameter) (C), a collector for the size fractions (D), and a gooseneck connector (E). The air jet inlet is threaded on the inside to receive an accurately bored nozzle.

The U-shaped sample tube oscillates approximately 200 times per minute under the action of leather-tipped fingers (F) mounted on a

motor-driven shaft. These oscillations are not free, but are constrained by the action of an abutment and spring. The action is such as to cause translatory-rotary contact between the flour sample and the air, which is highly efficient for the deflocculation action of the jet. The action prevents a haphazard shaking of the sample, which would be detrimental.

The cones of the settling chambers are tapped by a centrifugal tapper (G), so as to speed the downward movement of oversized material. These tappers consist of a pair of hammers rotating freely on

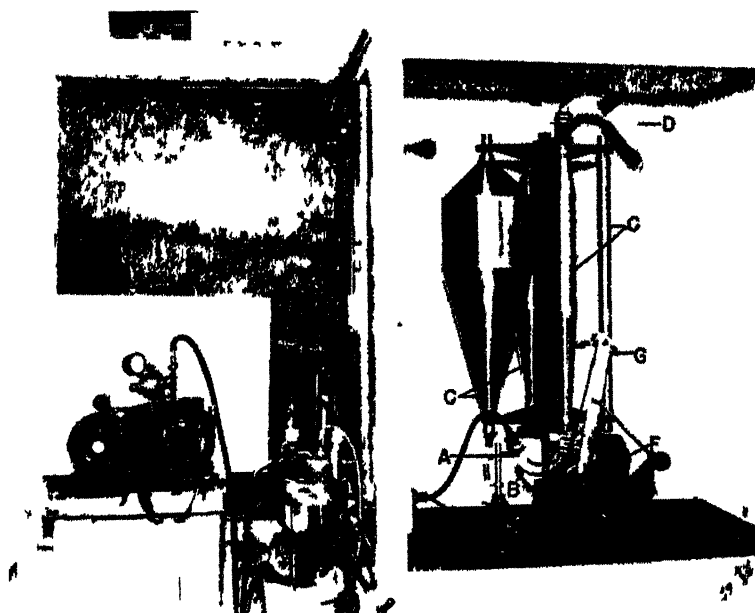


Fig 3. Roller Particle Size Analyzer

a shaft that is belted to the main motor shaft. The entire system is grounded to remove static electricity.

The apparatus has a carefully calibrated flowmeter (H). The entire range of air flow for flour is covered by two capillaries (I), which are interchangeable by means of a large bore three-way stopcock. A mercury manometer (J) measures the pressure drop across the inlet nozzle and provides a means for obtaining a flowmeter correction. This correction is applied in order to retain a constant pressure within the U-shaped sample tube for all air velocities.

Since the instrument was used as a "clean-up" measure on the particle size fractions obtained by sieving, the air velocity used was

such that it would remove particles up to the lower size limit of each fraction. Small particles removed comprise broken agglomerates, starch granules adhering to the larger flour particles, and all bran chips. The resulting flour fractions were of a well-defined particle size and were completely freed from any material other than pure endosperm particles of a stated size range. A comparison of the fraction particle sizes is shown in Figure 4. Microscopic observations were made as a size control on all of the fractions.

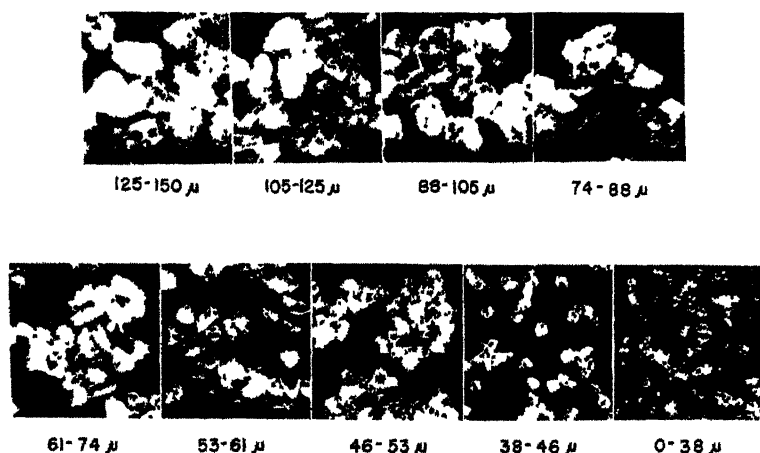


Fig. 4. Photomicrographs of the flour particle size fractions. Magnification: 35 X.

*Chemical and Physical Analysis.* Analysis for ash, protein, and gas production was carried out by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941). The specific gravity of each fraction was determined by employing the pycnometer and method described by Sharp (1927), with air buoyancy corrections applied in each case. The amylograph was used as described by Anker and Geddes (1944), while the farinogram curve patterns were obtained in the usual manner. The valorimeter was used as described by Johnson, Shellenberger, and Swanson (1946) to determine the curve characteristics. The National-Swanson-Working recording dough mixer was used for obtaining the mixogram curve patterns. Johnson, Swanson, and Bayfield (1943) have fully discussed the theoretical and practical application of the mixogram to cereal research.

Each flour fraction was baked using the baking procedure employed by Johnson, Swanson, and Bayfield (1943), except that the absorption was determined by use of the farinograph. The rich formula was used.

## Results and Discussion

Table I gives a summary of data for the relation of particle size to the various tests.

TABLE I  
RELATION OF FLOUR PARTICLE SIZE TO ASH, PROTEIN, AND AMYLOGRAPH,  
MIXOGRAPH, AND FARINOGRAPH CURVE CHARACTERISTICS<sup>1</sup>

Particle size range	Ash	Protein	Amylograph maximum viscosity	Mixograph		Farinograph	
				Area under curve	Mixing time	Absorption	Valorimeter reading
$\mu$	%	%	Frabender units	cm. <sup>2</sup>	min.	%	units
Flour	0.44	11.1	695	77.4	3	61.6	78
125-150	0.44	9.7	—	—	—	—	—
105-125	0.32	10.0	1020	71.7	3 $\frac{1}{2}$	58.9	74
88-105	0.35	10.4	935	72.3	3 $\frac{1}{2}$	60.3	77
74-88	0.36	10.6	880	72.3	3 $\frac{1}{2}$	60.1	77
61-74	0.37	11.4	875	74.2	3 $\frac{1}{2}$	60.6	76
53-61	0.39	12.3	830	79.4	3 $\frac{1}{2}$	61.7	66
46-53	0.43	13.5	735	83.8	3 $\frac{1}{2}$	64.3	74
38-46	0.52	13.7	—	—	—	—	—
0-38	0.51	9.1	570	65.0	4 $\frac{1}{2}$	61.5	57
0-105 <sup>2</sup>	0.44	11.1	695	76.2	3 $\frac{1}{2}$	62.1	82
38-150 <sup>3</sup>	0.41	11.9	695	74.6	3 $\frac{1}{2}$	62.1	80
38-105 <sup>4</sup>	0.41	12.0	845	79.2	3 $\frac{1}{2}$	62.1	85

<sup>1</sup> All values expressed on a 14% moisture basis.

<sup>2</sup> Flour with only the largest particles (105-150  $\mu$ ) removed.

<sup>3</sup> Flour with only the smallest particles (0-38  $\mu$ ) removed.

<sup>4</sup> Flour with the largest and the smallest particles removed.

**Ash.** A decrease in the size of the endosperm particle is accompanied by an increase of ash content of the particles. Since the particle size fractions have been subjected to air elutriation, where the removal of bran is completed, the resulting ash of the flour fractions is apparently an inherent characteristic of the particle in the fraction. The largest flour particles should then have the lowest ash content. This one fraction, however, contained such large chips of bran that an air velocity great enough to remove the bran would also have removed the flour particles. The 0-38  $\mu$  fraction is below the size of most existing flour particles. Thus it is composed largely of free starch granules and constitutes approximately 27% of the whole flour. Since the ash content of this fraction is high, it has a strong influence on the final ash content of the original flour.

**Spectrographic Analysis.** A spectrographic quantitative analysis for the elemental distribution as related to particle size is shown by the data in Table II.

TABLE II

RELATION OF FLOUR PARTICLE SIZE TO INORGANIC ELEMENTAL DISTRIBUTION<sup>1</sup>

Particle size range	Ash	Milligram percent in flour							
		P	K	Na	Ca	Mg	Mn	Fe	Cu
$\mu$	%								
Flour	0.44	79.0	70.2	6.6	16.2	14.9	3.0	1.0	0.2
125-150	0.44	70.1	68.8	11.0	16.2	13.2	2.4	1.9	0.8
105-125	0.32	68.5	75.9	6.1	11.8	10.3	1.9	0.7	0.5
88-105	0.35	58.6	36.2	8.1	10.8	6.7	1.2	0.4	0.6
74-88	0.36	57.4	39.9	6.8	11.7	13.1	2.1	0.4	0.8
61-74	0.37	87.8	39.9	6.6	13.9	12.8	2.5	0.7	1.2
53-61	0.39	84.7	35.8	3.5	15.6	12.7	2.5	1.0	1.2
46-53	0.43	76.2	40.3	6.1	14.9	12.1	1.9	1.0	2.1
38-46	0.52	91.5	67.6	5.7	16.3	17.2	3.3	1.1	2.5
0-38	0.51	78.2	61.8	6.1	14.6	12.3	2.2	0.5	2.4

<sup>1</sup> All values expressed on a 14% moisture basis.

Potassium and phosphorus are the major constituents in the ash. Phosphorus is high in the particle size ranges of 0-74 and 105-150  $\mu$  and low for 74-105  $\mu$ . Potassium is high in the 0-38 and 105-150  $\mu$  particle sizes and low in the intermediate size material. These two elements do not appear to follow a trend of distribution according to the quantity of ash or particle size, although a lower quantity is seen in the intermediate size material. Small differences are seen for the other inorganic elements in distribution in the various fractions, although no trend is noted.

**Protein.** The relation of particle size to protein content is the same as with ash. A decrease in the size of the endosperm particle is accompanied by an increase of the protein content. The 0-38  $\mu$  fraction contains some free starch granules, which accounts for its low protein content.

Morris, Alexander, and Pascoc (1946) removed various zones of endosperm from the wheat kernel by using a dentist drill and found that the innermost zone produced a flour whose ash and protein contents were lower than any other zone. As the zones radiated out toward the bran coat, the flours increased in their ash and protein contents. Thus, the largest flour particles originated in the innermost zone of the endosperm, while succeeding smaller particles came from zones as they radiated out toward the peripheral layer. Apparently the innermost zone was more vitreous, remaining in larger particles. These larger particles were lower in protein content than smaller flour particles. However, Berg (1947) found that the vitreousness of the endosperm and the subsequent flour particle size does not depend upon the protein content.

*Gas Production.* The relation of gas production to the fractions having malted wheat flour added and to untreated fractions is shown in Table III. The production of gas was least on the untreated frac-

TABLE III  
RELATION OF FLOUR PARTICLE SIZE TO GAS PRODUCTION

Particle size range	Gas production in mm. of mercury							
	Untreated				1% malted wheat flour added			
	4	6	8	24	4	6	8	24
$\mu$	hrs	hrs	hrs	hrs	hrs	hrs	hrs	hrs
Flour	306	333	366	535	499	595	666	1050
125-150	—	—	—	—	—	—	—	—
105-125	209	222	238	339	381	442	500	928
88-105	221	238	252	352	408	473	523	846
74-88	232	251	267	379	415	481	533	896
61-74	224	241	255	362	408	473	523	874
53-61	227	242	257	351	418	476	536	889
46-53	250	266	284	386	440	501	557	891
38-46	290	309	330	447	470	545	602	926
0-38	417	462	506	682	495	697	821	1141
0-105 <sup>1</sup>	320	351	385	561	503	603	672	1055
38-150 <sup>2</sup>	242	267	277	383	422	480	532	840
38-105 <sup>3</sup>	243	265	279	387	433	505	555	861

<sup>1</sup> Flour with only the largest particles (105-150  $\mu$ ) removed

<sup>2</sup> Flour with only the smallest particles (0-38  $\mu$ ) removed.

<sup>3</sup> Flour with the largest and the smallest particles removed

tion whose particles were largest in size. With a decrease in the size of the flour particles an accompanying increase in gas production was obtained. This same relation existed when the fractions were supplemented with malted wheat flour. Since the increase in height of these curves is nearly all proportionate over the curves given by the unmalted fractions, there is no indication of a concentration of amylase in any one fraction. The difference in gas production is apparently due to the susceptibility of the flour particles to enzyme attack. With a decrease in size of the flour particle, the susceptibility of the particle becomes greater. The 0-38  $\mu$  fraction shows the highest rate of gas production in both untreated and malted fractions. This is probably due to a concentration of starch granules, some of which are ruptured and therefore are highly susceptible to amylase action.

*Amylograph Curves.* The relation of particle size to the maximum viscosity determined by the amylograph is shown by data in Table I. The viscosity is least for the smallest particle size fractions. With an increase in size of the flour particles there is an accompanying increase of the viscosity.

There was no evidence from either gas production data or amylograph curves of the differential influence of amylase activity in any one fraction as compared with another. Thus the marked differences in the viscosity curve heights are due to the various flour particle sizes. The 0-38  $\mu$  fraction is composed of many starch granules, some of which are ruptured from mechanical operation. Because of the increased surface area thus created, the amylase brings about the degradation of the starch granules more rapidly. The liquefying action of amylase is in opposition to the viscosity increase caused by gelatinization.



Fig 5 Comparison of the bread baked from the various particle size fractions.

*Farinogram and Mixogram Curves.* The relation of particle size to farinogram and mixogram data is given in Table I. Only small differences between curves are noted except for the 0-38  $\mu$  fraction. Since the protein content of a flour is one of the most important factors in determining the farinogram and mixogram patterns, the low protein content of the 0-38  $\mu$  fraction accounts for the poor curve produced. The mixograms gradually increase in height as the particle size decreases, as shown by the area under the curves. This curve rise is attributed to a protein increase. There is also a general trend for the water absorption to increase with a decrease in the size of the particle. This can be attributed to an increased protein content and to the greater amount of surface area exposed as particle size decreases. The valorimeter value for each curve showed little relation to particle

size; however, on the flours from which the coarse material, fine material, and both coarse and fine material had been removed, the value of the curve is increased greatly.

*Specific Gravity.* The basis upon which the air elutriation principle is founded is that the same specific gravity exists for all particle sizes of flour. Thus a change of air velocity is required to remove the different-sized particles having a uniform specific gravity. Careful determination of the specific gravities of all particle sizes showed very little difference among them.

*Baking Results.* A comparison of the loaves of bread baked from the particle size fractions is shown in Figure 5 and a summary of the baking data is given in Table IV.

TABLE IV  
RELATION OF FLOUR PARTICLE SIZE TO BAKING DATA

Particle size range	Loaf volume	Crumb color <sup>1</sup>	Texture grain <sup>2</sup>	External appearance (break and shred)
$\mu$	ml.	score	score	
Flour	760	75cy	83-o	Good
125-150	—	—	—	—
105-125	775	84cy	78-o	Poor, half shell top
88-105	775	85cy	81-c	Poor, half shell top
74-88	775	88cw	82-c	Poor, half shell top
61-74	795	90cw	85-o	Fair, partially shell top
53-61	860	90cw	88-o	Good
46-53	920	88cw	90-o	Good
38-46	—	—	—	—
0-38	610	78cy	75-o	Very poor, half shell top
0-105 <sup>3</sup>	785	78cy	86-o	Fair to good
38-150 <sup>4</sup>	805	80cy	87-o	Fair to good
38-105 <sup>5</sup>	835	80cy	88-o	Fair to good

<sup>1</sup> Crumb color—cy = creamy yellow, cw = creamy white.

<sup>2</sup> Texture-grain—c = close; o = open.

<sup>3</sup> Flour with only the largest particles (105-150  $\mu$ ) removed.

<sup>4</sup> Flour with only the smallest particles (0-38  $\mu$ ) removed.

<sup>5</sup> Flour with the largest and the smallest particles removed.

Perhaps the most important characteristic sought in a loaf of bread is loaf volume, assuming that the loaf grain and texture are good. Since loaf volume is directly related to the protein content of the flour, the loaf volume is low for the coarse flour fractions whose protein content is low. An increase in loaf volume is obtained as the protein content increases, or as the flour particle size decreases.

The largest particles had the lowest protein content, and produced shell-topped bread of poor volume and appearance. The smallest flour particles produced bread of largest volume with good grain and texture and excellent color. The larger flour particles produced bread with a creamy colored crumb, which indicates a higher concentration of the carotinoid pigments.



The flours with the coarse, fine, and coarse and fine particles removed produced loaves superior to those obtained from the original flour, and compared favorably with the 46–53  $\mu$  fraction whose high protein content produced the best loaf.

*Practical Implication of the Study.* Wheat tempering or conditioning must influence to a large extent the granularity of a flour because of the existing relation of particle size and zonal origin.

Air separation of stock in the milling process can assume a greater role for the selection of flour endosperm of improved quality. Air separation can eliminate the starchy, low protein, high ash material which tends to lower the baking quality of a flour.

### Summary

A commercial hard wheat flour was fractionated by means of wire sieves and by air elutriation into 12 different ranges of flour particle sizes. Chemical and physical tests performed upon the various fractions showed a wide range in characteristics, which were related to the size of the flour particles. An increase of ash, protein, water absorption, gassing power, area under mixogram curves, and loaf volume was found, with a decrease in the size of the flour particles to the lowest limit of approximately 38  $\mu$ . The 0–38  $\mu$  fraction size was composed of some free starch granules with a slight overlap of the very finest flour particles, resulting in a fraction of low protein, low viscosity, reduced area under mixogram curve, and less loaf volume. This same fraction was high in ash and gassing power. No significant trend for inorganic elemental distribution as related to the quantity of ash or particle size was noted.

Protein content was the dominant factor influencing the quality of each fraction of the flour, and the ash content was shown to be an inferior yardstick of flour quality measurement.

Gas production measurements indicated that amylase activity was practically uniform throughout the various flour fractions and that gas production increased with decrease in particle size.

The carotinoid pigments are apparently concentrated in the coarser flour particles, since the finer particles produced a bread loaf whose color was creamy white.

Removal of the finest and the coarsest particles from flour enhanced greatly the remaining portion of the sample. The improved quality is not due to the more uniform granulation of the flour, but to the increased protein content of the sample, which results from the removal of the fine and coarse particles.

The specific gravities remained the same for all particle size fractions.

Baking results for each fraction were closely related to flour quality as determined by the various physical and chemical tests.

The relationship of particle size to ash and protein is indicative of the zonal source of these particles.

### Acknowledgments

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# SPECIFIC SURFACE OF WHEAT FLOURS.

## I. DETERMINATION BY AIR PERMEABILITY METHOD

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A knowledge of the amount of surface associated with a given weight of a finely divided material has been of importance in studying various processes in a large number of industries. It is possible that this same type of knowledge may be of value to the cereal chemist in checking various milling operations and in correlating specific surface with other properties of a flour.

During the last decade two methods of determining the specific surface of finely divided materials have been developed. Low temperature adsorption isotherms, using nitrogen gas primarily as the adsorbate, have been used in determining surface areas of porous and finely divided materials by Emmett and Brunauer (1937), Brunauer, Emmett, and Teller (1938), and Harkins and Jura (1944). The other method is based upon the permeability of porous media to fluid flow. Lea and Nurse (1939), Gooden and Smith (1940), Blaine (1941), and Pechukas and Gage (1946) have all successfully used air permeability methods for measuring surface areas of powders.

The purpose of this work was to develop a method for the determination of the specific surface of cereal flours that would have a high experimental accuracy and would be adaptable for routine analysis. From consideration of the experimental technique of measurement, the air permeability method was chosen as the one most likely to achieve this purpose. A comprehensive study of the experimental factors affecting the determination of specific surface was made including the following variables: pressure differential, method of determining rate of flow of air, porosity, cross-sectional area of sample tube, and length of sample tube.

The theory of the permeability method and details of experiments conducted to test its validity have been discussed by Carman (1937, 1938, 1939) and lately reviewed in detail by Sullivan and Hertel (1942).

Experimentally the method determines the rate of flow of a fluid through a cylindrical plug of a porous material.

The specific surface can be expressed by the equation:

$$S_v = \frac{14}{d_s} \sqrt{\frac{A \Delta P t}{Q \eta L}} \cdot \frac{\epsilon^3}{(1 - \epsilon)^2} \quad (1)$$

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where

$S_v$  = specific surface of the powder in sq. cm. per gram

$d$  = density of the powder

$A$  = cross-sectional area of the powder in sq. cm.

$L$  = thickness of the powder in cm.

$\Delta P$  = pressure difference driving the fluid through the medium in grams per sq. cm.

$Q$  = volume of air in ml flowing through the powder in time  $t$ , expressed in seconds

$\eta$  = viscosity of the fluid in poises

$\epsilon$  = porosity =  $1 - \frac{W}{dAL}$

$W$  = weight of powder in grams.

Carman has shown for certain materials that for the same powder at different porosities the porosity function,  $\epsilon^3/(1 - \epsilon)^2$ , is reasonably accurate over a fairly wide range of porosities. To see if this were true in the case of a single wheat flour a number of determinations were made at different porosities. Rearranging equation (1) into the following form:

$$\frac{\epsilon^3}{(1 - \epsilon)^2} = \frac{S_v^2}{K^2} \cdot \frac{\eta}{\Delta P t} \quad (1a)$$

where

$$K = \frac{14}{d_s} \sqrt{\frac{A}{QL}}$$

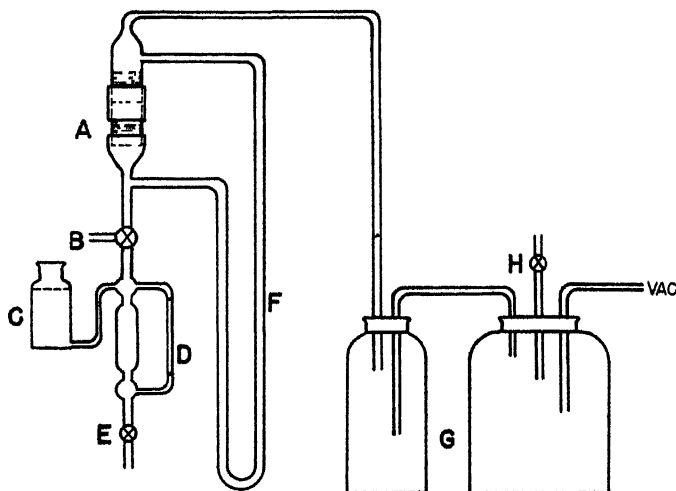
and by plotting the porosity function  $\epsilon^3/(1 - \epsilon)^2$  vs.  $\eta/\Delta P t$  a straight line should result passing through the origin with slope  $S_v^2/K^2$ .

Results from the experiments on wheat flour have indicated that equation (1a) is not correct in its present form.

### Apparatus for Determining Specific Surface

The essential features of the apparatus developed for determining the specific surface of wheat flour are shown in Figure 1. The apparatus consists of a vacuum source connected to two surge bottles G and regulated by pinch clamp H which establishes a pressure differentially measured by kerosene manometer F across the cylindrical sample tube A. Ten machined brass sample tubes ranging in diameter from 0.4 cm. to 1.2 cm., and in length from 2.0 cm. to 4.0 cm., have been used in the apparatus. The volume of each tube was determined by filling with mercury and weighing. The length of the tube was determined by use of a micrometer. By dividing the volume by the length, the average cross-sectional area of the tube was calculated.

Various methods have been described in the literature to measure the pressure head across the sample plug and to determine the volume of air passing through the plug in a given length of time. One of the simplest forms of apparatus described employs an open end manometer to measure the pressure differential, and a pipet dipping into a flask containing water to measure the volume of air. Use of such an apparatus was found to be unsatisfactory because the level of water drawn into the pipet acted as an additional arm of the manometer, and the



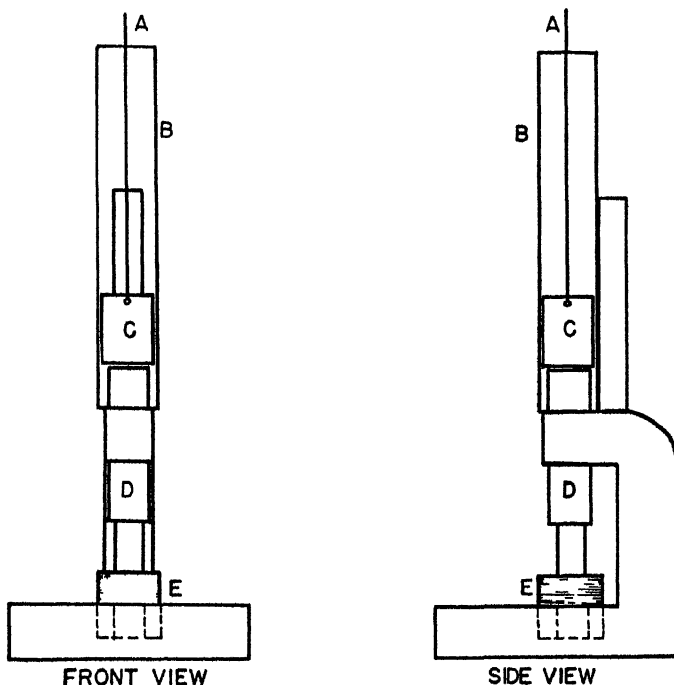
### AIR PERMEABILITY APPARATUS

Fig. 1. Air permeability apparatus. (A) sample tube, (B) three-way stopcock, (C) kerosene reservoir, (D) volume measurement device, (E) two-way stopcock, (F) kerosene manometer, (G) surge bottles, (H) vacuum regulator.

pressure determination was consequently dependent upon the volume measurement. In order to avoid this difficulty a direct-connecting manometer F, Figure 1, was used. The volume of air was determined by apparatus BCDE. With the three-way stopcock B open to the air, a pressure differential is established across A. After steady flow has been attained, usually in three minutes, B is closed and air from system D is drawn through A. As the pressure in D is lowered, kerosene from C flows into D replacing the air. The kerosene level in C is adjusted so that a lowering of pressure approximating 1 mm. of kerosene in D causes the liquid in C to flow into chamber D. Thus the pressure measurement by manometer F is not affected appreciably by the volume measurement. The rate of flow of air through A is determined by measuring the time that it takes for the kerosene to fill the space in container D, defined by two etch marks in a capillary

side arm. This volume in the apparatus used is 4.977 ml. The capillary side arm is used because of the greater ease of reading the kerosene meniscus. When the determination is complete the kerosene is drained from D by stopcock E and returned to reservoir C.

To achieve uniform porosity throughout the length of a sample tube a special tamping apparatus, Figure 2, is used. A sample tube



### TAMPING APPARATUS

Fig. 2 Tamping apparatus. (A) wire handle, (B) glass guide tube, (C) bucket containing lead shot, (D) steel plunger, (E) machined sample tube

is screwed into the bottom plate E and a plunger of outside diameter 0.002 inch less than the inside diameter of the sample tube is placed in the tamping apparatus. A small amount of flour, 0.08–0.10 g., is added to the tube and a weighted bucket C is dropped a given distance through glass tube B, hitting the plunger D. By changing the weight of C, the distance through which it falls, or the number of times it drops, a tube may be packed to any desired uniform porosity. The weight of the flour is determined by weighing the sample tube before and after filling.

The sample tube is then placed in the permeability apparatus and a constant pressure differential established. After three minutes the

volume measurement is made and the actual pressure differential is read at the same time. If desired, the rate of flow of air can be determined at several different pressures. The temperature of the room is observed after each determination so that the correct density of kerosene and also viscosity of air may be read from appropriate tables or curves.

Density of a flour is determined by methods described by Bauer (1945) using a 25 ml. pycnometer, xylene as the liquid, and a temperature of 25°C. For most of the flours investigated to date  $d$ , varies between 1.42 to 1.45 grams/ml. Because of the small difference observed in the densities of the flours investigated to date in this laboratory, it is felt that density determinations need only be made on those samples that have either abnormally high or abnormally low water contents. No appreciable change in density has been observed in flours that have widely different specific surfaces, i.e., density is not a function of particle size. A value of 1.45 is used for  $d$ , for most routine samples and only in special cases is a separate density determination made.

### Study of Experimental Factors

To test any possible effects due to different pressures, a series of tests was made using differential pressures ranging from 0.2 cm. to 100 cm. of kerosene pressure. By holding all other factors constant the product  $\Delta Pt$  should be constant throughout the pressure range. This was found to be the case within an experimental error of 1%.

Using a patent flour made from hard winter wheat, a comprehensive study of the effect of the size of the sample tubes was made. Ten different tubes were used, the smallest being 20 mm. long and 4 mm. diameter (designated as Tube 20-4), and the largest 40 mm. long and 12 mm. diameter (Tube 40-12). Calculations based on equation (1) gave a value of the specific surface of approximately 2,000 sq. cm. However, there was a standard error of 10% of a single determination from the mean among the 50 determinations made using the 10 different sample tubes.

By adding a correction constant,  $b$ , to equation (1a) an equation results which satisfies the data to a better degree:

$$\frac{\epsilon^3}{(1 - \epsilon)^2} = \frac{S_w^2}{K^2} \cdot \frac{\eta}{\Delta Pt} + b. \quad (2)$$

Figure 3 shows the data for Tube 40-12 plotted according to equation (2). All 10 tubes gave similar plots, indicating that the porosity function  $\epsilon^3/(1 - \epsilon)^2$  is not a sole function of porosity,  $\epsilon$ . The

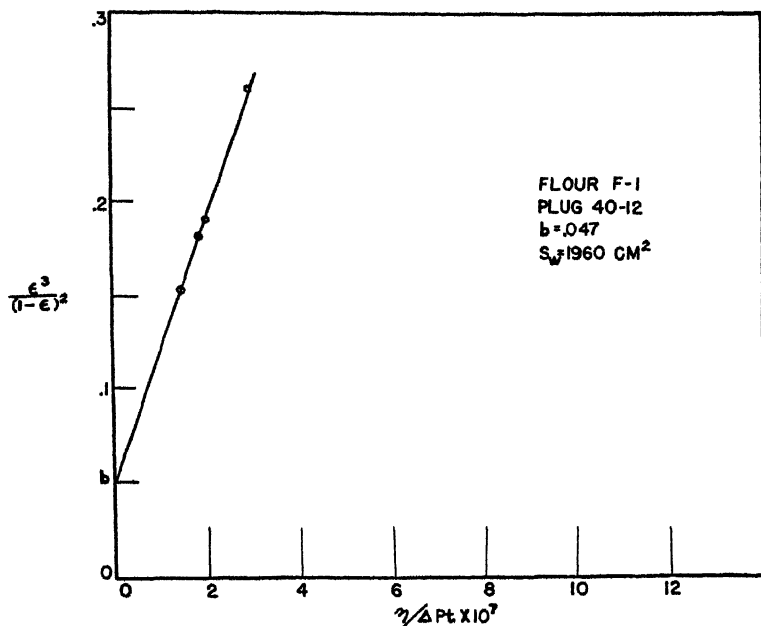


Fig. 3. Experimental plot using equation (2).

values of  $S_w$  for the different tubes along with the values of the intercept,  $b$ , are presented in Table I. The standard error in  $S_w$  is only 2.6%.

TABLE I  
SPECIFIC SURFACE OF PATENT FLOUR CALCULATED BY  
USE OF EQUATION (2)<sup>1</sup>

Tube	$S_w$ cm. <sup>2</sup>	$b$
20-4	2120	0.030
20-5	2020	0.036
20-6	1950	0.035
20-8	1990	0.044
20-10	2020	0.040
20-12	2080	0.034
30-6	2020	0.039
30-12	2010	0.037
40-6	2170	0.036
40-12	1960	0.047
Average	2035	0.038

$$^1 \frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S_w^2}{K^2} \frac{\eta}{\Delta P} + b.$$

The values of  $S_w$  calculated from (2) are dependent upon the dimensions of the sample tube as is shown in Figure 4. A minimum in the value of  $S_w$  was found for a sample tube having a cross-sectional area of 0.40 cm. and length of 2 cm., or a value of  $\sqrt{A/L}$  of approxi-



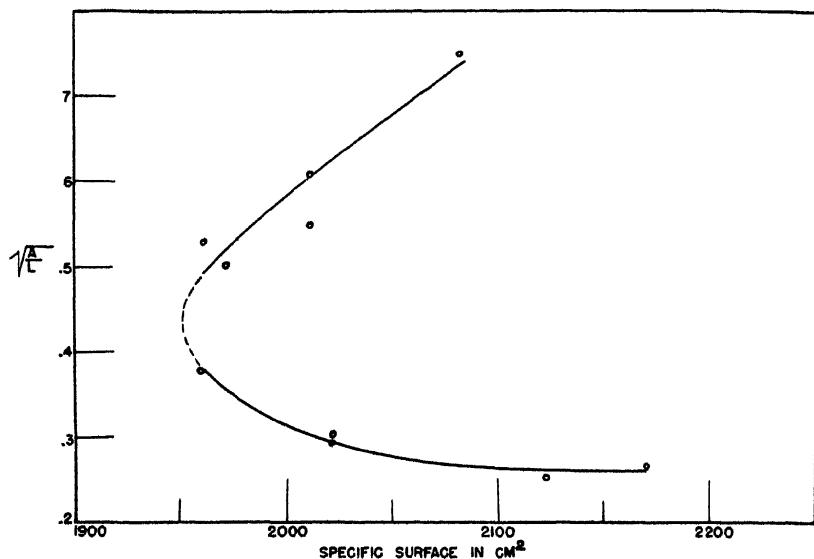


Fig. 4. Specific surface of patent flour (F-1) as a function of the dimensions of sample tube based on calculations from equation (2).

mately 0.45. This indicates that  $S_w$  is dependent upon some function involving  $A$  and  $L$ . Accordingly, equation (2) was modified so that all terms involving  $A$  and  $L$  were transposed to the left side, giving:

$$\frac{A}{L} \cdot \frac{\epsilon^3}{(1 - \epsilon)^2} = S_w^2 \cdot \frac{d_s^2 Q \eta}{14^2 \Delta P t} + C, \quad (3)$$

where  $C$  is a correction term.

By plotting  $A\epsilon^3/L(1 - \epsilon)^2$  vs.  $d_s^2 Q \eta / 14^2 \Delta P t$  the slope will be  $S_w^2$  and intercept  $C$ .

TABLE II  
SPECIFIC SURFACE OF PATENT FLOUR CALCULATED BY  
USE OF EQUATION (3)<sup>1</sup>

Tube	$\sqrt{\frac{A}{L}}$	$S_w$ cm. <sup>2</sup>	$C$
20-4	0.253	2170	0.0016
40-6	0.264	2180	0.0018
20-5	0.297	2010	0.0032
30-6	0.305	2020	0.0038
20-6	0.373	1980	0.0047
20-8	0.504	1960	0.0115
40-12	0.530	1980	0.0138
30-12	0.612	1960	0.0175
20-10	0.616	1950	0.0200
20-12	0.745	1950	0.0340
Average of tubes 20-6 to 20-12		1963	

$$\frac{A}{L} \cdot \frac{\epsilon^3}{(1 - \epsilon)^2} = S_w^2 \cdot \frac{d_s^2 Q \eta}{14^2 \Delta P t} + C.$$

Figure 5 shows the data for Tube 40-12 plotted according to equation (3). All 10 tubes gave similar plots. The corresponding values of  $S_w$  and  $C$  for the various tubes are given in Table II. The values of  $S_w$  decrease with increasing values of  $\sqrt{A/L}$ , see Figure 6, until a value of  $\sqrt{A/L}$  of .373 is reached, after which  $S_w$  is independent of  $\sqrt{A/L}$  within  $\pm 1\%$  variance.

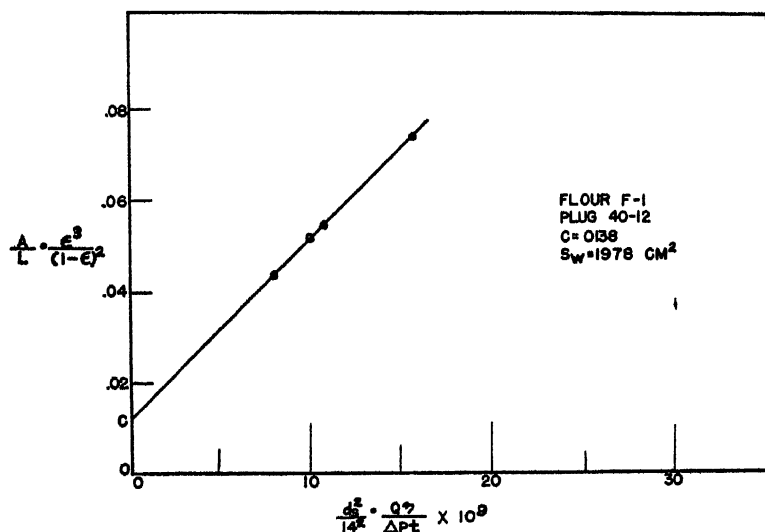


Fig. 5. Experimental plot using equation (3).

Considering the results presented in Figure 6, it is postulated that a small layer of flour adjacent to the walls of the sample tube is inactive and probably very little air will flow through this layer. If this is true, sample tubes of small cross-sectional area or of extreme length would give values of  $S_w$  that were too high. However, if the cross-sectional area of the sample tube were increased or the length decreased, the percentage effect of an inactive layer would gradually decrease. This postulate needs to be verified by studies of other materials and a comprehensive review of the dynamics of fluid flow through powder beds.

Experimental reproducible results with a standard error of 0.5% can be obtained in the case of wheat flour by using equation (3) for the calculation of  $S_w$ , provided that the ratio of  $\sqrt{A/L}$  of the sample plug is greater than 0.4. At least two determinations are necessary by this method to find  $S_w$ . In order to evaluate  $S_w$  by one determination the function of  $S_w$  vs.  $C$  must be known. This function was determined for Tube 20-8 and is plotted in Figure 7. The value of

$C$  for sample Tube 20-8 increased as the specific surface of a flour decreased. To make a corrected calculation of  $S_u$  from one experimental determination, an approximate  $S_u$  is found using equation (1) and by use of Figure 7 the value of the  $C$  term is estimated. Then substituting this value of  $C$  into equation (3) a corrected value of  $S_u$  may be obtained.

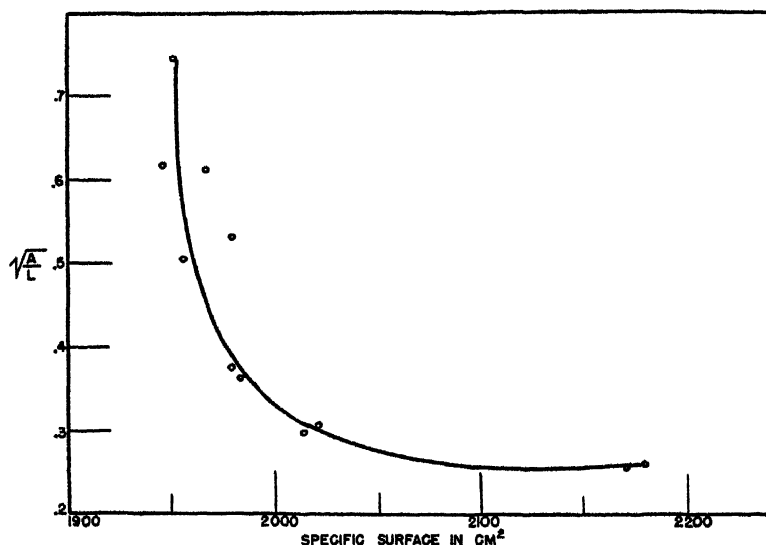


Fig. 6. Specific surface of patent flour (F-1) as a function of the dimensions of sample tube based on calculations from equation (3).

Since no previous values of the specific surface of flour have been presented in the literature, a calculation of the particle size diameter has been made assuming that the flour particles are smooth spheres. The relation between  $S_w$  and  $D$ , the corresponding diameter of equivalent smooth spheres, is:

$$D = \frac{6}{S_w d_s} \quad (4)$$

Comparison of equivalent particle diameters with the results of sedimentation experiments of Kent-Jones, *et al.* (1939, 1941) and Hildebrand, Ferrari, Borchardt, and Anker (1942) indicates that the equivalent diameters obtained from surface area measurements are smaller than those obtained either by sedimentation methods or by microscopic observations. This discrepancy is undoubtedly due to the fact that both the sedimentation and microscopic values do not take into account the irregular shape of the flour particles. The jagged appearance of flour particles is readily seen microscopically and this

irregularity of the surface will increase the surface area. Consequently any equivalent diameter calculation based on equation (4) will be smaller in value. This conclusion is borne out by the data of Emmett (1942) on zinc oxide pigments. He found that the values of equivalent diameter determined by air permeability and adsorption methods agreed very closely, but diameters obtained by direct microscopic count were twice as large. Practically, the surface area calculations are more significant than equivalent diameter calculations, but

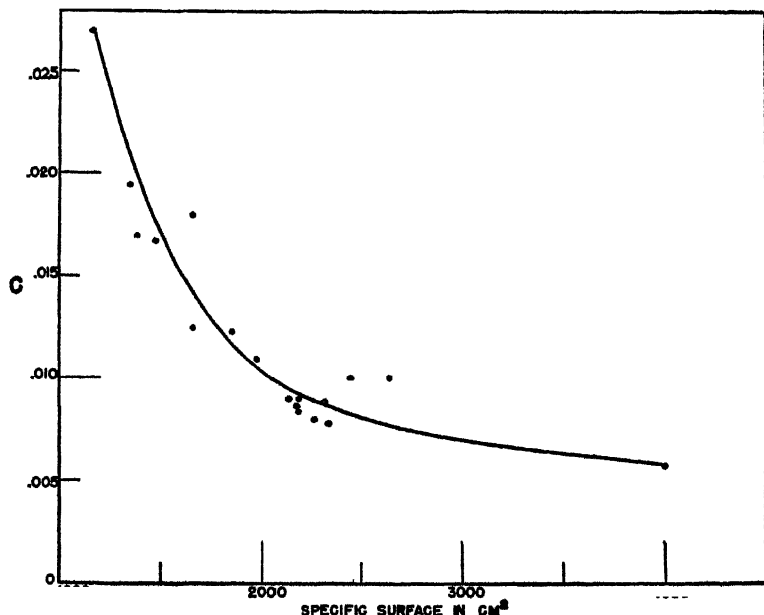


Fig. 7. Variation of correction term C with specific surface. Sample tube 20-8.

for comparison with previous results equivalent diameter calculations are presented in Tables III, IV, and V.

### Results and Discussion

Determinations of the specific surface of fractions of Patent flour held on wire sieves and on silk bolting cloths have been made as well as determinations of the specific surface of 45 mill streams from hard red winter wheat. Specific surface determinations of the fractions of flour held on various ASTM wire sieves are presented in Table III. The  $S_w$  value for the overs of ASTM 100 mesh is slightly greater than the value for ASTM 140 mesh. This is probably due either to the short length of time of the shaking process or to agglomeration of some of the flour particles. The total surface area for each flour fraction

TABLE III

SPECIFIC SURFACE DETERMINATIONS ON FRACTIONS OF PATENT FLOUR  
OBTAINED BY USING ASTM WIRE SIEVES  
(IN RO-TAP SHAKER FOR 10 MINUTES)

ASTM sieves	Weight, grams	Specific surface, $S_w$ in cm. <sup>2</sup>	Diameter, $D$ , in microns	Total surface area, grams $\times S_w$
Over 80	0.136	—	—	—
Over 100	10.958	1380	29.9	15120
Over 140	8.239	1350	30.6	11100
Over 180	5.339	1660	24.9	8850
Over 300	23.400	2440	16.85	57300
Under 300	0.459	—	—	—
Total	48.531			92370

Total exclusive of "over 80" and "under 300" = 47.936.

$S_w = \frac{92370}{47.936} = 1,930 \text{ cm.}^2$ , the average specific surface from above sieve analysis

$S_w = 1,960 \text{ cm.}^2$ , the average specific surface as determined on original flour.

is given in the fifth column of Table III. The sum of these surface areas divided by the total weight gives a value of  $1,930 \text{ cm.}^2$ . The value of  $S_w$  of the original flour was  $1,960 \text{ cm.}^2$ . This agreement is a good substantiation of the experimental technique of determining  $S_w$ .

TABLE IV

SPECIFIC SURFACE DETERMINATIONS ON FRACTIONS OF PATENT FLOUR  
OBTAINED BY USING SILK BOLTING CLOTHS  
15 MIN. (ROTARY MOTION)  
(50 GRAMS TOTAL)

Silk	Weight, grams	Specific surface, $S_w$ in cm. <sup>2</sup>	Diameter, $D$ , in microns	Total surface area, grams $\times S_w$
Over 9XX	0.334	—	—	—
Over 12XX	2.016	1480	28.0	2975
Over 15XX	19.028	1160	35.5	22190
Over 20 std.	11.790	2140	19.3	25180
Over 25 std.	13.485	2640	15.6	35600
Under 25 std.	2.888	4000	10.3	11550
Total	49.541			97495

Total exclusive of 9XX = 49.237.

$S_w = \frac{97495}{49.237} = 1,970 \text{ cm.}^2$ , the average specific surface from above silk analysis.

$S_w = 1,960 \text{ cm.}^2$ , the average specific surface as determined on original flour.

The same type of results presented in Table IV were obtained using silk bolting cloths. The flour over 12 xx silk had a higher value of  $S_w$  than the fraction held on 15xx silk, indicating either too short a shaking time or agglomeration. The weighted surface area calculated

from these various samples agreed within 1% of the value of  $S_w$  determined on the original flour.

The results of the specific surface studies on 45 mill streams are presented in Table V. The flour from the break rolls shows a considerable variation in specific surface. The flour from 3 Break N has a low specific surface which correlates with the low diastatic activity of that particular mill stream. The Cuts stream has the lowest

TABLE V  
SPECIFIC SURFACE OF MILL STREAMS

Stream	Moisture %	Ash %	Protein %	Diastatic activity, <sup>1</sup> mg. maltose	Specific surface, $S_w$ in cm. <sup>2</sup>	Diameter, D, in microns
1 Break N	16.6	0.57	11.8	143	2130	19.3
S	16.5	0.57	11.7	138	2000	20.6
2 Break N	16.3	0.50	12.0	138	1960	21.0
M	16.4	0.51	12.2	128	1940	21.2
S	16.5	0.52	12.2	138	2043	20.2
3 Break N	16.4	0.50	13.1	98	1670	24.6
S	16.2	0.51	13.2	118	2142	19.2
4 Break N	15.7	0.57	14.3	133	2190	18.8
S	15.6	0.57	14.4	133	2178	18.9
5 Break N	15.4	0.69	15.5	133	2230	18.5
S	15.1	0.69	15.5	133	2170	19.0
1 Siz N	15.0	0.47	10.8	304	2240	18.4
S	15.0	0.47	10.7	323	2185	18.8
2	14.6	0.50	11.1	308	2500	16.5
B	15.3	0.58	11.5	158	1975	20.9
Germ	14.4	0.65	11.1	287	2720	15.2
1 Tail	14.1	0.58	11.0	355	2835	14.5
2	13.7	0.59	10.8	388	2690	15.3
3	12.7	0.91	12.8	298	2902	14.2
1 Low Grade	13.2	0.62	12.0	269	2270	18.2
2	12.9	0.56	11.6	292	2440	16.9
3	12.3	0.71	11.8	317	2340	17.6
Bran and						
Shorts N	15.0	1.03	15.2	178	2450	16.8
S	15.0	1.03	15.2	178	2550	16.2
Patent	14.6	0.42	11.0	294	2162	19.0
Clear	14.3	0.75	13.1	365	2380	17.3
1 Mids N	14.7	0.38	10.8	306	2332	17.7
E	14.7	0.38	10.8	312	2315	17.8
S	14.3	0.37	10.7	306	2438	16.9
2 Mids N	14.4	0.38	10.6	269	2180	18.9
M	14.6	0.38	10.7	269	2260	18.2
S	14.3	0.38	10.6	275	2190	18.8
3 Mids N	14.4	0.41	10.7	253	2185	18.8
S	14.3	0.39	10.7	248	1855	22.2
4 Mids N	13.5	0.39	10.6	275	2217	18.6
S	13.5	0.39	10.6	275	1982	20.8
5 Mids	13.6	0.44	11.2	253	1762	23.4
6 Mids	13.3	0.46	11.1	296	2260	18.2
7 Mids	13.3	0.48	11.5	275	2222	18.5
Cuts	16.2	0.45	12.2	103	1435	28.8

<sup>1</sup> Total maltose after diastasis for one hour. Method 20.61 of Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.

specific surface of any of the flours tested. The Germ, Tailings, Low Grades, Bran, and Shorts streams have relatively high specific surfaces. As indicated by these results the various streams that are blended to produce Patent flour vary as much as 20% in specific surface values.

### Summary

A rapid method for the determination of the specific surface of wheat flours by the air permeability is presented. A critical study of several experimental factors influencing the reproducibility of results has been made. The size of the sample tube is critical, the best results being obtained with tubes having dimensions such that the square root of the ratio of cross-sectional area to length is greater than 0.4. It has been found necessary to revise the air permeability theory in order to reduce the dependency of specific surface on the porosity function.

Patent flours, made from hard winter wheat, have been found to have a specific surface of about 2,000 cm.<sup>2</sup>. The specific surface of various sieve fractions of such a flour indicate that it is composed of material having a specific surface of 1,200 to 4,000 cm.<sup>2</sup>. Determinations on 45 mill streams indicate that the streams blended to produce Patent flour vary as much as 20% in specific surface values.

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## CHARACTERIZATION OF WHEAT GLUTEN. I. PROTEIN-LIPID COMPLEX FORMATION DURING DOUGHING OF FLOURS. LIPOPROTEIN NATURE OF THE GLUTENIN FRACTION

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Several investigators have observed that gluten, as it is ordinarily obtained from flour, contains 5 to 10% lipids (Dill, 1925), and that only a small fraction of these can be extracted with petroleum or ethyl ether (Dill, 1925; Fisher and Halton, 1933; Blish, 1936; Sullivan, 1940; and others). McCaig and McCalla (1941) suggested that this protein-lipid complex may be formed when the dough is made. The experiments to be described here confirm this concept in part. In addition, it has been possible to demonstrate that mere wetting of flour causes binding of a considerable part of the lipids. Furthermore, fractionation studies with gluten have shown that most of the lipid is associated with the "glutenin," rather than the gliadin, portion. These findings suggest that glutenin, as it occurs in gluten, but not necessarily in the wheat grain, should be considered a *lipoprotein*.

### Materials and Methods

The flour used in most of the experiments was an unbleached bakers' flour, containing 10.5% moisture and, on a dry basis, 0.4% ash and 15.3% protein (nitrogen  $\times$  5.7).

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The extractability of the lipids from flour, dried doughs, and glutens was determined on samples which had been thoroughly dried in a vacuum oven at 60°. Total lipids were determined by extraction with absolute alcohol in the Soxhlet apparatus, followed by evaporation of the alcohol and resolution of the lipid fraction in chloroform. Only the chloroform-soluble fraction was weighed. That such extraction was essentially complete was demonstrated as follows: A weighed portion of gluten was hydrolyzed with 10% potassium hydroxide, acidified, and extracted with ether. The ether-soluble fraction represented the fatty acids plus the unsaponifiable fraction of the gluten lipids. Its weight was in good agreement with a similar fraction obtained from the alcohol extract of the same gluten sample (see also Tucker, 1946).

Ether-soluble fractions were obtained (1) by Soxhlet extraction, (2) by centrifugation and decantation of several portions of solvent, and (3) by percolation of the solvent through a column of the material. Results obtained by the latter two methods were in agreement and gave more consistent, but slightly lower, results than did Soxhlet extraction.

In some cases flours were worked up into doughs by hand. In others, doughs were prepared as follows: 15 g of the flour and 15 ml of water were placed in a Waring Blendor bowl. The motor of the Blendor was connected to a varying resistance so that it could be run at a very low speed. Doughs of uniform consistency were readily obtained in very short mixing times.

Doughs, glutens, and protein solutions were dried by lyophilization, that is, they were frozen and the water was removed by sublimation in a high vacuum. The dried products had a porous structure and were readily reduced to fine powders, in contrast to the hornlike materials so often obtained when gluten is dried by other techniques. This particular method of handling materials is perhaps the most important advance over previous investigations. The fineness of the dried powders lent increased confidence in the validity of extraction data.

#### **Effect of Wetting and Doughing on the Solubility of Flour Lipids in Ether**

Approximately 70% of the total lipids of the flour could be extracted with ether. Similar data have been reported by other investigators (cf. Sinclair and McCalla, 1937; Sullivan and Howe, 1938; Barton-Wright, 1938; Tucker, 1946).

A sample of the flour was wet with water in such manner as to avoid any mechanical doughing action. The flour was added, in small amounts at a time, to an excess of water that was kept stirred. No

clumps formed. The mixture was quickly frozen and dried. Only 40% of the lipids could now be ether-extracted.

An attempt was made to determine the least amount of water that would cause such a loss in solubility. A sample of the flour was placed in a closed vessel over water (100% relative humidity). Toluene vapor was present to prevent mold or bacterial growth. At the end of three weeks, the sample was found to contain 20% moisture. It was then dried (vacuum oven, 60°) and extracted with ether. As with the original flour, 70% of the total lipids were extractable. It may be concluded that the very high moisture contents that occasionally occur under adverse storage conditions would probably not cause additional gluten-lipid combination.<sup>2</sup>

TABLE I  
EXTRACTABILITY OF LIPIDS OF FLOUR

	Of flour	Of total lipids
	%	%
Alcohol-extractable fraction	1.45	100
Ether-extractable fraction (EEF)	1.0	70
EEF—flour stored at 20% H <sub>2</sub> O content	1.0	70
EEF—flour brought to 30% H <sub>2</sub> O content	0.57	39
EEF—flour wet, then dried	0.55	38
EEF—flour doughed, then dried	0.09	6

Another sample of the flour was brought to 30% moisture content in the following manner: The calculated amount of powdered ice was thoroughly mixed with a previously cooled sample of the flour at -34.4°C. After one month at -9.4°C, during which time most of the ice sublimed into the flour, the mixture was brought to room temperature. It then formed a very stiff dough. The dough was carefully broken into small pieces and dried by lyophilization. In this sample, only 40% of the total lipids were extractable by ether. These preliminary observations indicate that the critical moisture level for the combination, or "binding," of lipids is between 20 and 30%, and that, if mechanical doughing is avoided, an approximately equal amount of the flour lipid (30%) becomes bound whether a minimum amount or an excess of water is added.

Portions of the flour were made into doughs by the Waring Blendor technique, then dispersed in excess water or dilute acetic acid. The dispersions were frozen and dried. Now only 6% of the lipids could be extracted with ether.

These observations (summarized in Table I) show that approximately 30% of the lipids of the particular wheat flour used are bound,

<sup>2</sup> The free lipid to bound lipid ratio of 2:1 is typical for high protein flours. It does not necessarily reflect the ratio in the wheat endosperm. The results of the experiments described in this report suggest the possibility that some changes in the state of the lipid fraction might occur during storage or milling operations.

that is, they cannot be extracted with ether. When the flour is wet with water, another 30% becomes bound, and when it is kneaded into a dough, a third 30% becomes bound. The bonds holding the lipid in combination are formed in the presence of water, and broken in the presence of alcohol.

### Capacity of Flour to Bind Lipids

Samples of flour containing excess flour lipids were prepared as follows: A petroleum ether extract of the flour was added in varying quantities to separate aliquots of the unextracted flour. After most of the solvent had evaporated, the last traces were removed in a vacuum oven (60°). These samples, which now contained up to 8.5% total lipids, were made into doughs by the Waring Blendor technique, dispersed in excess water, frozen, and dried. The dried doughs were powdered, then extracted with ether by the centrifugation and decantation method. The results (Table II) demonstrate clearly that an

TABLE II  
BINDING OF EXCESS LIPID BY FLOUR DURING DOUGHING

Sample	Total lipid in dried dough	Lipid extractable by ether	Bound lipid	Bound lipid as percent of total lipid
	%	%	%	%
1 <sup>1</sup>	1.45	0.10	1.35	93
2	2.30	0.25	2.05	89
3	3.14	0.34	2.80	89
4	4.82	0.64	4.18	85
5	8.42	4.31	4.09	49

<sup>1</sup> No added lipids.

amount of lipid up to about three times that in the original flour can be bound so that it is no longer available for ether extraction, but that this amount apparently approaches the capacity of the flour.

### Preferential Binding of Phospholipids

It has been assumed that the phosphorus content of the lipid fractions represents the phospholipids present. The results of phosphorus analyses obtained during the experiment just described are shown in Table III. They indicate that even when four times the original amount of phospholipid is present, only 3% can be extracted by ether, in contrast to the half of the total lipids so removable.

In a separate experiment, it was found that the ether extract of a flour that had been wetted and then dried contained 30% of the total lipid but only 4% of the total lipid-phosphorus. The ether extract of the original flour contained 70% of the total lipid and 30% of the lipid-

TABLE III  
PRELIMINARY BINDING OF PHOSPHOLIPID

Sample	Lipid phosphorus present in dried dough	Phosphorus extracted with ether as per- cent of total phosphorus	Bound lipid phosphorus	Bound lipid as percent of total lipid
	%	%	%	%
1	0.007	0	100.0	93
2	0.010	0	100.0	89
3	0.013	0.4	99.6	89
4	0.018	1.7	98.3	85
5	0.030	2.8	97.2	49

phosphorus. Thus, during the wetting procedure, half of the ether-soluble lipids but almost 90% of the ether-soluble phosphorus became bound. Pending confirmation in detail, these observations suggest that phospholipids are bound preferentially, compared to other constituents of the flour lipids.

The marked differences in extractability between total lipid and lipid-phosphorus, before and after doughing, may be taken as additional evidence that the changes observed are real and not due to mechanical interference with the extraction procedure.

### Participation of Gluten

The experiments described so far have dealt with changes in the amounts of lipids extractable by ether from flours. The role of the flour proteins in these phenomena remains to be elaborated.

Fisher and Halton (1933) observed that considerable amounts of lipids added to flours may be retained in the gluten washed from them. Sullivan (1940) states, "When gluten is washed out from flour it contains over half of the lipids of the flour. The gluten must first be treated with alcohol in order to liberate the lipids, since only a trace can be removed by direct extraction with ethyl ether or petroleum ether." (Cf. also Dill, 1925.) Our experiments confirm such observations. With the flour at our disposal, approximately 70% of the total lipids could be accounted for in the gluten fraction after thorough washing. (Fisher and Halton, 1933, record 70%.) The dried gluten contained 7.4% total lipids, approximately one-third of which were extractable with ether. The validity of the extraction data was demonstrated as follows: One sample was dried directly from the frozen state; another was dispersed completely in dilute acetic acid, then frozen and dried. The results of extraction procedures with these samples were in agreement. These results and the observations of previous workers indicate that the binding of lipid in flour is largely a function of the gluten.

In order to determine which of its components is involved in this reaction, we have fractionated gluten by several techniques introduced by previous investigators (see Blish and Sandstedt, 1929; Spencer and McCalla, 1938; Blish, 1936). In each case the bulk of the lipids was found to follow those fractions usually designated by the term "glutenin."

One fractionation was accomplished briefly as follows: Gluten was dispersed in dilute acetic acid, as first suggested by Blish and Sandstedt (1926). Excess starch and a small amount of insoluble protein were removed by passing the solution through a Sharples centrifuge. Proteolytic enzymes were destroyed by a brief heat-treatment (Olcott *et al.*, 1943).<sup>3</sup> The glutenin fraction was separated by adjusting the solution to pH 5.0, and purified by several resuspensions in solutions of pH 5 to 6, followed by centrifugations. The combined supernatant solutions were brought to pH 6.8 and the precipitate separated. This precipitate was stirred with water containing acetic acid to pH 5.5, then again centrifuged. The insoluble fraction is referred to as the "middle fraction" in Table IV. The soluble portion was considered to

TABLE IV  
DISTRIBUTION OF LIPIDS IN GLUTEN FRACTIONS

	Fraction recovered	Lipid content	Lipid as percent of total lipid
Glutenin	% 46	% 20.0	% 81.5
Middle fraction	13	11.2	13.0
Gliadin	41	1.5	5.5

be mostly gliadin. All preparations were dialyzed and dried by lyophilization. The results of extraction experiments are shown in Table IV. Very little of the total lipids were extractable with ether. Alcohol disrupted the bonds that held the lipid to the protein (cf. Blish, 1936; Sullivan, 1940). Such properties are similar to those of many protein-lipid complexes now recognized as occurring widely in nature (see Chargaff, 1944). They suggest that glutenin, as it occurs in wheat gluten, should be considered a lipoprotein.

Several years ago, Blish (1936) called attention to the presence of a protein-lipid complex in gluten, and suggested that the portion of wheat gluten other than gliadin was composed of a mixture of glutenin and the complex. Small fractions containing amounts of lipid ranging up to 44% were isolated. So far we have not obtained fractions containing more than 15-20% lipids (from normal flour), nor have we been

<sup>3</sup> Enzyme activity is destroyed by exposure of the gluten solution in dilute acetic acid to a temperature of 90°-100° for as short a time as 30 seconds (Olcott and Sapirstein, unpublished experiments).

able to differentiate between glutenin and the lipoprotein complex. Until this is accomplished, it appears preferable to identify glutenin with the lipoprotein complex.

It is of interest that Dill in 1925 described a preparation of glutenin in which nonaqueous solvents had been avoided. The product contained 11% total lipids, and only 3% ether-extractable lipids. The significance of his observation has escaped attention.

### Discussion

The recognition that the lipid and the protein of wheat flour form a lipoprotein complex *during* the wetting and doughing of flour may help in the unraveling of some of the, as yet, little understood aspects of bread technology.

Some of the difficulties hitherto encountered in attempts to isolate and characterize the components of gluten may be attributed to the unrecognized lipoprotein nature of glutenin. Many investigators have agreed on the properties of gliadin (Larmour and Sallans, 1932; Blish, 1945), a protein that is apparently not involved in lipid binding. Few have agreed on the properties of glutenin. The use of alcohol or alkali in its preparation disrupted the lipoprotein complex and irreversibly changed its properties (see Sinclair and McCalla, 1937).

The existence of *glutenin* as an entity is debatable, and therefore even the use of the term is of doubtful propriety. Nevertheless, it is serviceable as a name for the "nongliadin-like fraction" of wheat gluten. For future use we suggest "lipoglutenin" to indicate this protein moiety as it exists in gluten, and "glutenin" for the same fraction from which the lipids have been extracted. These designations conform to the terminology suggested by Chargaff (1942, 1944) for the egg yolk proteins, "lipovitellin" and "vitellin."

Experiments along the lines indicated in this paper are now being applied to other types of flours, and the results will be presented in detail in subsequent publications.

### Summary

A high-protein patent flour contained 1.5% total lipids, 70% of which were extractable with ether. The flour was mixed with water with a minimum of doughing, then dried by lyophilization. Only 40% of the lipids were now extractable with this solvent. After the flour was kneaded into a dough and dried, less than 10% of the lipids could be extracted.

The capacity of the flour to "bind" lipids during wetting and doughing was ascertained by determining the extractability of added flour lipids. At least three times the amount of lipid normally present

could be bound by the doughing procedure. Phospholipids were bound preferentially.

Most of the lipid bound was associated with the gluten, rather than with the nonprotein, constituents of flour; and, when gluten was fractionated, the lipid was found to be bound to the "glutenin," rather than to the gliadin fractions.

Glutenin fractions containing up to 20% lipids have been obtained. It is proposed to call the nongliadin fraction of gluten, "lipoglutenin." The term "glutenin" should be reserved for fat-free preparations.

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# RELATION OF SUSCEPTIBLE STARCH, ALPHA-AMYLASE, AND SUGARS ORIGINALLY PRESENT TO FLOUR GASSING POWER

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The correlation between gas production and diastatic activity has been studied by Larmour, Geddes, and Whiteside (1933), Sandstedt and Blish (1934), Graesser (1936), Munz and Bailey (1936), Blish, Sandstedt, and Mecham (1937), Davis (1938), Davis and Tremain (1938), Singh and Bailey (1940), and Hildebrand and Geddes (1940). Some found a high correlation between the two measurements; others found it to be barely significant. Fisher, Halton, and Hines (1938) obtained a significant correlation with the total gas only and concluded that the one-hour maltose test could not be used as a safe substitute for the gas production test. All these observers sought a significant correlation between gas produced and diastatic activity, while making no correction for the effect of the fermentable sugars present originally in the flour.

Blish, Sandstedt, and Astleford (1932) appear to have been the first to realize that the original fermentable sugars present in the flours are of considerable importance in predicting their gassing power. They found that the reducing sugar originally present was low in amount, varied little, and could be disregarded, but that the sucrose content varied from 1.00–1.74% and was sufficient to account for the few discrepancies in the gas production of individual flours as predicted from the diastatic activity. Sandstedt (1934) compared the sucrose and the maltose produced by diastasis with the gas pressure produced during a four-hour period and considered that there was a satisfactory agreement between the results obtained by the two methods.

Davis and Worley (1934) found a linear relationship between the diastatic activity and gassing power of 122 flours and calculated the regression equation and the error of predicting one value from the other. They found that the sucrose content was the major factor accounting for the fact that some samples did not fall very close to the average line of relationship. Eva, Geddes, and Frisell (1937) found, on the other hand, that although the correlation between diastatic activity and gassing power was highly significant, it was not sufficiently high to predict the latter from the former with accuracy. The closest relationship was obtained for the 30-minute period pre-



coding the critical time. Gas production after the critical time was not closely associated with maltose values based on the customary conditions of diastasis, which suggests that such maltose values are conditioned largely by the quantity of the more diastatically susceptible hexosans (dextrins and ruptured granules) present. Increasing the time or temperature of diastasis would therefore be expected to give a truer index of the ability of a flour to support gas production after the critical period. The results obtained by using optical methods, in which the sucrose content is taken into account, for estimating diastatic activity suggested that, while the correlations were not significantly higher than those involving the maltose value, in a more extended series employing more suitable conditions of diastasis these methods might prove superior for the purpose of estimating flour gassing power.

Davis (1938) found that, although the correlation between the gas-producing ability of flours and their diastatic activity values was highly significant, it was difficult to place the flours in order of their gas-producing capacity. Differences in sucrose content accounted for the greater part of the variation. Shellenberger (1938) pointed out that a fair degree of accuracy may be expected if the diastatic activity is used to predict the gassing rate of a flour after the original sugar has been fermented. Sandstedt and Blish (1938) observed that adjustment of flours by addition of sucrose, so as to give equal dough sugar levels, does not ensure equal rates of gas production throughout fermentation. They attributed the differences to a variable content in the flours of a maltose fermentation activator.

The importance of factors other than the original sucrose content of flours in determining the correlation between autolytic diastatic figures and gassing figures has been investigated by several workers. Sandstedt, Blish, Mecham, and Bode (1937) found that some flours had a higher gassing power than could be accounted for by a knowledge of their maltose figures and their sucrose content, and they ascribed this to the presence of a high content of an enzyme responsible for rendering raw starch available to beta-amylase. Such an enzyme was known to occur in sound wheat flour. Blish, Sandstedt, and Kneen (1938) applied a yeast manometric method to the evaluation of malt supplements and found that the gassing responses of different malts (in the first few hours) fell in the same order as did their alpha-amylase values.

Sandstedt (1938), working with a series of flours also investigated by Davis and Tremain (1938), found a very high correlation between their alpha-amylase values and their gassing and autolytic maltose values. The highest correlation was between alpha-amylase and

gassing power. Two malts were encountered in which the gassing power was low, although the alpha-amylase was high. He suggests that the other factor concerned might be a separate enzyme responsible for raw starch saccharification, and that it registers in this gassing method but not in any of the other methods. Bottomley (1938) did not find a good correlation between diastatic activity and gassing power. The discrepancies between actual gassing power and that expected from a knowledge of the diastatic activity and sucrose content were not uniform.

Kneen and Sandstedt (1941) have given a further explanation of the significance of alpha-amylase in determining gassing power in sugar-deficient doughs. This enzyme was found to be responsible for hydrolyzing raw starch and also, in combination with beta-amylase, it carries hydrolysis much further than does the latter enzyme alone. In 1942 they obtained further evidence of the high correlation between the gassing power response caused by malts and their alpha-amylase activity.

Meredith, Eva, and Anderson (1944) showed that the relationship between gas stimulation by malted wheat flours and their alpha-amylase activity occurs both within and between wheat varieties. Their correlation coefficient is not as high as that reported by Kneen and Sandstedt (1942), who stated that determinations of added gassing power and of alpha-amylase appear to be equally reliable for evaluating malts. Gas production in the corresponding unmalted flours was assumed to be correlated with their alpha-amylase content, although this was not determined in the case of the sound wheat flour. Hildebrand and Geddes (1940) have shown that if care is taken to select the proper levels of diastatic activity and gas production, malt dosage may be estimated from either with equal precision, although the use of diastatic activity is much less convenient.

It seems from the foregoing review that the factors to be considered in estimating the potential gas production of a flour are the fermentable sugars originally present and the amount of raw starch-splitting amylase. No measurement has been made of the importance of variation in the starch substrate as a factor in gas production. A fair degree of satisfaction in the estimation of potential gas production has actually been attained by the use of a knowledge of the sucrose content and the diastatic activity of a flour. It has been shown by Dadswell and Gardner (1947) that at least two factors, the alpha-amylase content and the susceptibility of the starch substrate, are intimately related to the diastatic activity figure of a flour. Thus it is reasonable to postulate that the substrate factor should also be considered in gas production.

By using values which measure each factor separately, a better understanding of the role of each in potential gas production should be possible. The simplest combination is the amount of alpha-amylase as represented by the dextrin formed during autolysis at 62°C, the susceptibility of the starch substrate as measured by the maltose due to beta-amylase, and the amount of sugars originally present in the flour as represented by the total of the sucrose and maltose originally present. Comparison between the gas production as estimated on the basis of the diastatic activity figure together with the quantity of sugars originally present and that calculated on the basis of the individual factors concerned is of interest as a means of judging how satisfactorily gas production can be estimated by either method, and whether any advance has been made in the accuracy of estimation by use of the separate factors.

In order to make further observations on the influence of variations in the substrate on gas production, a series of flours having a uniform amylase and sucrose content but a variable substrate on which the amylase could act and representing at the same time different varieties and places of growth would be useful, but such a series is difficult if not impossible to obtain. However, some information could be gained by substituting a uniform concentration of alpha- and beta-amylase for the variable concentration in a series of natural flours and observing the effect of variations in the susceptible starch and in the sucrose content on the gas production.

### Notation

Notations for the various gas production figures reported in this paper are similar to those used, for corresponding determinations of sugars and diastatic activity, by Dadswell and Gardner (1947). Since both sets of notations are used in the present paper, the full list is given below:

- R = Reducing sugars originally present in flour.
- Z = Sucrose originally present in flour.
- T = Total sugars originally present in flour, i.e.,  $R + Z$ .
- $G_T$  = Gas production due to total sugars (T).
- S = Susceptible starch in flour.
- $G_S$  = Gas production due to susceptible starch.
- $M_\beta$  = Maltose formed by excess  $\beta$ -amylase.
- $G_\beta$  = Gas production due to excess  $\beta$ -amylase.
- $M_\alpha$  = Maltose formed by excess  $\alpha$ -amylase.
- $G_\alpha$  = Gas production due to excess  $\alpha$ -amylase.
- $A_g$  = Gross autolytic diastatic activity.

- $G_g$  = Gas production corresponding to above.  
 $A_n$  = Net autolytic diastatic activity.  
 $G_n$  = Gas production corresponding to above.  
 $D$  =  $\alpha$ -amylase in flour by dextrose method.  
 $G_1$  = Gas production during first 3 hours.  
 $G_2$  = Gas production from end of 3rd to 7th hour.  
 $G_3$  = Gas production during first 5 hours  
 $G_4$  = Total gas production.

The determinations were made on natural flours and on a corresponding set of artificial flours (see previous paper). Notations for natural flours are given above; notations for artificial flours are similar but a prime mark is added, e.g.,  $G_T'$ ,  $M_\beta'$ ,  $G_1'$ , etc.

### Materials and Methods

**Materials.** The preparation of crude alpha- and beta-amylase was carried out as previously described by Dadswell and Gardner (1947). Amylase-free flour for use in gassing experiments was prepared by allowing 10 g. of flour mixed with 6 ml. of 0.25 *N* hydrochloric acid to stand at room temperature for 30 minutes. One ml. of 7% sodium carbonate was then added and the dough was well mixed.

**Total sugars originally present in flour ( $T$ ).** Sucrose ( $Z$ ) was estimated according to the method of Sandstedt (1937). The reducing sugars originally present ( $R$ ) were determined as described by Dadswell and Gardner (1947). The sum of these amounts represent total sugars originally present in the flour ( $T$ ).

**Measurement of gassing power.** The total gas pressure in mm. of mercury was measured according to the method of Sandstedt and Blish (1934) in a pressuremeter similar to that described by them. The mean internal capacity of the pressuremeters was  $275 \pm 1.8$  ml.

Two sets of results are available for each sample, those based on the natural flours (i.e., on naturally occurring amylases) and those based on the artificial flours (i.e., on the combined action of 60 mg. of crude alpha-amylase and 60 mg. of crude beta-amylase on the amylase-free flour). For the artificial flours, the dry powdered amylases were added to the amylase-free flour followed by 1 ml. of a 30% suspension of bakers' yeast. Fermentation was carried out as for natural flours in a pressuremeter.

The gas pressure produced has been considered in relation to time under four headings: that produced during the first 3 hours being designated as  $G_1$  for the natural flours and as  $G_1'$  for the artificial flours; that produced from the end of the third to the end of the seventh hour as  $G_2$  and  $G_2'$ ; that produced during the first 5 hours as

$G_3$  and  $G_3'$ ; and finally the total gas pressures  $G_4$  and  $G_4'$ , which were the maximum readings obtained.

The total gas pressure results from the fermentation of the sugars originally present in the flour and of those sugars produced as a result of amylase action. The sugars produced by the amylases, corresponding to the gross diastatic activity and denoted by  $A_g$  or  $A_g'$ , are designated when fermented as the gas pressures  $G_g$  and  $G_g'$ . These sugars were further divided into those due to beta-amylase ( $M_\beta$ ) and those due directly or indirectly to the presence of other amylases ( $A_n$  and  $A_n'$ ). The corresponding gas pressures were therefore designated as  $G_\beta$ ,  $G_n$ , and  $G_n'$ . The experimental details concerned in differentiating the gas produced from these various classes of sugars are given below.

*Gas pressure due to sugars originally present in flour ( $G_T$ ).* Ten grams of flour, treated to render it amylase-free, were fermented by 1 ml. of 30% yeast suspension in a pressuremeter. The total pressure developed was designated as  $G_T$ , which corresponds to the total sugar originally present ( $T$ ).

*Gas pressure due to susceptible starch.* (1) Gas due to beta-amylase ( $G_\beta$ ). Ten grams of flour, treated to render it amylase-free, were mixed with an excess of crude beta-amylase, in this case 120 mg. The total pressure developed was that due to the fermentation of the sugars originally present in the flour and of the maltose formed as a result of the action of beta-amylase on the susceptible starch. Therefore the total pressure developed minus  $G_T$  equals  $G_\beta$ , the pressure due to beta-amylase action.  $G_\beta$  has a fixed value for each flour since it is determined in the presence of an excess of beta-amylase and corresponds to the maltose due to beta-amylase ( $M_\beta$ ).

(2) Gas due to alpha-amylase ( $G_\alpha$ ). Ten grams of flour, treated to render it amylase-free, were mixed with 60 mg. of crude alpha-amylase. The total pressure developed was due to the fermentation of the sugars originally present in the flour and of the sugar formed as a result of the action of alpha-amylase. Therefore the total pressure developed minus  $G_T$  equals  $G_\alpha$ .

*Gas pressure due to combined action of amylases and susceptible starch.* (1)  $G_g$ , corresponding to the gross diastatic activity, represents the difference between the pressure due to the total gas produced ( $G_4$ ) and that due to the fermentable sugars originally present in the flour ( $G_T$ ), and is therefore the pressure due to fermentation of the sugars produced by the action of the naturally occurring amylases ( $G_4 - G_T = G_g$ ).

$G_g'$  corresponding to  $A_g'$ , the gross diastatic activity for artificial flours, is equal to  $G_4'$  minus  $G_T$ .

(2)  $G_n$ , for natural flours, represents the difference between the pressure due to the total gas produced ( $G_4$ ) and that due to both the fermentable sugars originally present in the flour ( $G_T$ ) and those formed by beta-amylase acting alone ( $G_\beta$ ), and corresponds to  $A_n$ , the net diastatic activity figure for natural flours ( $G_4 - G_T - G_\beta = G_n$ ).

For artificial flour  $G_n' = G_4' - G_T - G_\beta$ .

### Results and Discussion

Flours from the same six varieties of white winter wheat, each grown at three places in Victoria, Australia, as were studied in connection with diastatic activity (Dadswell and Gardner, 1947) were used in a study of gas production. The analytical data are recorded in Tables I and II.

TABLE I

SUCROSE, TOTAL SUGARS, AND GAS CORRESPONDING TO SUGARS AND TO GROSS AND NET DIASTATIC ACTIVITY VALUES FOR NATURAL AND ARTIFICIAL FLOURS

Sample number	Sucrose	Total sugars in original flour	Gas formed from total sugars in original flour	Gas formed by natural flours corresponding to:		Gas formed by amylase-free flour with added amylases			
						Excess beta-amylase	60 mg. crude alpha-amylase	Beta- and alpha-amylase corresponding to:	
				Gross diastatic activity	Net diastatic activity			Gross diastatic activity	Net diastatic activity
	Z	T	$G_T$	$G_g$	$G_n$	$G_\beta$	$G_\alpha$	$G_g'$	$G_n'$
	mg.	mg.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1	260	290	178	439	231	208	279	701	493
2	166	200	143	958	744	214	292	750	536
3	265	301	227	368	203	165	227	668	503
4	251	295	211	525	340	185	256	617	432
5	220	246	148	299	127	172	261	647	475
6	147	181	145	353	234	119	271	490	371
7	250	288	189	581	467	114	242	526	412
8	169	192	138	181	102	79	173	434	355
9	178	204	136	173	65	108	187	482	374
10	229	267	178	728	628	100	192	450	350
11	220	263	195	424	340	84	158	434	350
12	159	189	107	282	161	121	197	423	302
13	217	264	203	211	139	72	155	440	368
14	186	216	155	237	162	75	170	426	351
15	229	269	194	289	191	98	155	473	375
16	139	165	123	134	56	78	186	339	261
17	134	158	107	116	55	61	126	341	280
18	135	163	113	142	70	72	132	308	236

There are significant differences between place means and between variety means for sucrose (Z) and for total fermentable sugars (T). For gas due to the sugars originally present ( $G_T$ ), only the place means differ significantly (Table III). When uniform amounts of alpha-

TABLE II  
GAS PRODUCTION BY NATURAL AND ARTIFICIAL FLOURS

Sample number	Natural flours				Amylase-free flours with added beta- and alpha-amylases			
	First three hours	End of third to end of seventh hour	First five hours	Total gas	First three hours	End of third to end of seventh hour	First five hours	Total gas
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>1</sub> '	G <sub>2</sub> '	G <sub>3</sub> '	G <sub>4</sub> '
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1	233	108	293	617	308	333	520	879
2	216	291	395	1101	321	336	559	893
3	227	101	324	595	380	279	587	895
4	220	178	348	736	321	303	545	828
5	205	72	246	447	327	245	508	795
6	217	74	260	498	304	196	449	635
7	235	129	320	770	338	189	474	715
8	158	41	183	319	313	124	393	572
9	185	37	209	309	332	139	425	618
10	270	124	351	906	335	145	431	628
11	242	95	300	619	335	149	439	629
12	165	60	200	389	304	118	377	530
13	213	51	243	414	329	173	454	643
14	176	61	210	392	304	128	388	581
15	211	70	253	483	339	180	462	667
16	150	38	171	257	279	104	346	462
17	135	33	154	223	272	109	343	448
18	142	40	165	255	264	92	321	421

amylase were added to yeasted amylase-free doughs, there was a significant difference between the means for varieties and between means for places. Corresponding additions of beta-amylase gave significant differences between variety means only. The amount of gas formed corresponding to the gross diastatic activity for the natural flours ( $G_4 - G_T = G_R$ ) shows a significant variation between means of varieties and places, as does the equivalent value  $G_R'$  for the artificial flours, although the significance is of a higher order in the latter case. The variance in the amount of gas  $G_R$  corresponding to the net diastatic activity in the natural flours is significant for differences between means of varieties only, but in the case of the artificial flours the variance of the corresponding value ( $G_R'$ ) is highly significant for differences between means of varieties and places.

The gas formed from the artificial flours shows a highly significant difference between means of places for each of the four time intervals, and between means of varieties for each time interval other than the first three hours. For the natural flours, however, in which at least one other variable is present, namely, the amount of alpha-amylase, the results are not so uniform. The variance between means of places

TABLE III

ANALYSIS OF VARIANCE FOR THE EFFECT OF WHEAT VARIETY AND LOCATION OF GROWTH ON SUCROSE, TOTAL SUGARS, GAS FORMED IN DIFFERENT TIME INTERVALS, AND ON GAS CORRESPONDING TO TOTAL SUGARS AND TO GROSS AND NET DIASTATIC ACTIVITY VALUES FOR NATURAL AND ARTIFICIAL FLOURS

Source of variation	Degrees of freedom	Z	T	G <sub>T</sub>	G <sub>E'</sub>	G <sub>N'</sub>	G <sub>β</sub>	G <sub>E</sub>	G <sub>N</sub>	G <sub>α</sub>
Between means of varieties	5	2,391**	2,353 <sup>~</sup>	534	42,288**	15,452**	6,769**	97,978 <sup>~</sup>	69,471*	8,155**
Between means of places	2	9,554**	12,932**	9,083**	25,994**	16,136**	1,279	84,211*	67,794	1,939**
Remainder	10	410	518	291	1,896	998	498	19,846	17,898	223
		G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>1</sub> + G <sub>2</sub>	G <sub>1'</sub>	G <sub>2'</sub>	G <sub>3'</sub>	G <sub>4'</sub>
Between means of varieties	5	1,697 <sup>~</sup>	7,903 <sup>~</sup>	9,078**	55,184	15,032 <sup>~</sup>	530	16,951 <sup>~</sup>	12,970**	51,545**
Between means of places	2	5,675**	6,000	16,756**	262,394**	21,062**	3,506**	7,829**	14,638**	53,726**
Remainder	10	501	1,767	891	18,427	2,182	326	841	664	1,855

\* Significant.

\*\* Highly significant



is highly significant for the gassing periods up to the end of the fifth hour and for the total gas; and between means of varieties it is at least significant for periods up to the end of the seventh hour, but the variance between means of varieties for total gas and between means of places for the period from the fourth to the seventh hour is not significant.

*Correlations for Sugars Originally Present and the Gas Produced from Them.* The sugars originally present in the flours (T) correspond closely to the total gas produced from them ( $G_T$ ), as shown by the correlation of 0.927 (Table IV). Their relationship to the gas produced by the natural flours during the different time intervals varies over a wide range, the correlation (0.810) being highest for the first three hours ( $G_1$ ), but not even significant for the following four hours ( $G_2$ ). If the gas produced by the sugars originally present ( $G_T$ ) be compared with that produced in the different time intervals, the same condition holds, i.e.,  $G_1$  is the most and  $G_2$  the least closely related.

For the artificial flours, the correlation between T or  $G_T$  and the gas formed in the different periods is similar to that for the natural flours, the actual values of the corresponding correlation coefficients being slightly higher for the artificial flours.

TABLE IV  
CORRELATION COEFFICIENTS FOR TOTAL SUGARS AND GAS PRODUCTION

Natural flours			Artificial flours		
Variables correlated	$r$	$G_T$	Variables correlated	T	$G_T'$
$G_T$	0.927**	—	—	—	—
$G_1$	0.810**	0.774**	$G_1'$	0.780**	0.791**
$G_2$	0.356	0.334	$G_2'$	0.587*	0.524*
$G_3$	0.693**	0.675**	$G_3'$	0.740**	0.715**
$G_4$	0.542†	0.497*	$G_4'$	0.725**	0.655

*Susceptible Starch.* The sugars produced by beta-amylase activity ( $M_\beta$ ), and used to measure the susceptible starch, are closely correlated with the corresponding gas production ( $G_\beta$ );  $r = 0.947^{**}$ . Sugars produced by alpha-amylase activity, also used to measure susceptible starch, are not quite so closely correlated with gas production ( $G_\alpha$ );  $r = 0.826^{**}$ . The correlation between  $G_\beta$  and susceptible starch (S), as measured by the proportion of starch granules staining with iodine-congo red solution, though highly significant, is still lower;  $r = 0.754^{**}$ .

It has been shown by Dadswell and Gardner (1947) that there is a close relation between susceptible starch ( $M_\beta$ ) and both the gross ( $A_g$ ) and net ( $A_n$ ) diastatic activities. Gas productions corresponding to

gross and net diastatic activities (i.e.,  $G_g$  and  $G_n$ ) were therefore correlated with  $M_\beta$  as a measure of susceptible starch and with the gas corresponding to it ( $G_\beta$ ). The correlations are high for artificial

<i>Variables</i>	<i>Coefficient</i>	<i>Variables</i>	<i>Coefficient</i>
$G_g \times M_\beta$	0.577**	$G_g' \times M_\beta$	0.955**
$G_n \times M_\beta$	0.424	$G_g' \times G_\beta$	0.927**
$G_n \times M_\beta$	0.428	$G_n' \times M_\beta$	0.911**
$G_n \times M_\beta$	0.464	$G_n' \times G_\beta$	0.864**

flours, but for the natural flours the only coefficient that is significant is that for  $G_g \times M_\beta$ .

$M_\beta$  and  $G_\beta$  show a significant degree of correlation with the gas formed in the different periods in the natural flours and with that formed in all periods other than the first three hours in the case of the artificial flours (Table V). The values of the corresponding correlation coefficients are much higher for the artificial flours than for the natural flours in every period except the first three hours, when the reverse is the case. The closest relationship between susceptible starch ( $M_\beta$  or  $G_\beta$ ) and gas production is in the fourth to seventh hours ( $G_2$ ) for both the natural and artificial flours, and the least closely related was the first three hours of gas production ( $G_1$ ).

TABLE V  
CORRELATION COEFFICIENTS FOR GAS FORMED IN THE DIFFERENT PERIODS

Natural flours			Artificial flours		
Variables correlated	$M_\beta$	$G_\beta$	Variables correlated	$M_\beta$	$G_\beta$
$G_1$	0.519*	0.463*	$G_1'$	0.412	0.362
$G_2$	0.664**	0.726**	$G_2'$	0.948**	0.937**
$G_3$	0.676**	0.684**	$G_3'$	0.854**	0.836**
$G_4$	0.607**	0.632**	$G_4'$	0.918**	0.885**

Correlations were highest between amounts of sugar and gas pressures corresponding to the gross and net diastatic activity figures for the artificial flours, although the correlation coefficients for the natural flours were highly significant (Table VI). Both the net and gross diastatic activity figures were also closely correlated with the gas formed during all the different time intervals except the first three hours for the artificial and natural flours, the coefficients being higher for the former group. The gas pressures corresponding to the gross and net diastatic activity figures were in most cases more highly correlated with the gas formed during different time intervals than were the actual maltose values.

TABLE VI  
CORRELATION COEFFICIENTS RELATING TO COMBINED ACTION OF  
STARCH AND AMYLASES

Natural flours					Artificial flours				
Variables correlated	$A_g$	$G_g$	$\lambda_n$	$G_n$	Variables correlated	$A_g'$	$G_g'$	$\lambda_n'$	$G_n'$
$A_g$	—	0.821 <sup>++</sup>	—	—	$A_g'$	—	0.925 <sup>++</sup>	—	—
$A_n$	—	—	—	0.835 <sup>++</sup>	$A_n'$	—	—	—	0.925 <sup>++</sup>
$G_1$	0.577 <sup>++</sup>	0.716 <sup>++</sup>	0.517 <sup>+</sup>	0.699 <sup>++</sup>	$G_1'$	0.495 <sup>+</sup>	0.569 <sup>+</sup>	0.514 <sup>+</sup>	0.662 <sup>++</sup>
$G_2$	0.886 <sup>++</sup>	0.933 <sup>++</sup>	0.925 <sup>++</sup>	0.880 <sup>++</sup>	$G_2'$	0.976 <sup>++</sup>	0.961 <sup>++</sup>	0.967 <sup>++</sup>	0.925 <sup>++</sup>
$G_3$	0.834 <sup>++</sup>	0.920 <sup>++</sup>	0.819 <sup>++</sup>	0.875 <sup>++</sup>	$G_3'$	0.929 <sup>++</sup>	0.939 <sup>++</sup>	0.937 <sup>++</sup>	0.952 <sup>++</sup>
$G_4$	0.821 <sup>+</sup>	0.989 <sup>++</sup>	0.865 <sup>++</sup>	0.968 <sup>++</sup>	$G_4'$	0.951 <sup>++</sup>	0.976 <sup>++</sup>	0.944 <sup>++</sup>	0.979 <sup>++</sup>

Alpha-amylase as measured by dextrin formation (Kent-Jones and Amos, 1940) was not significantly correlated with gas production during the first three hours, but as the fermentation proceeded the effect of this enzyme became more apparent. The dextrin figure was significantly correlated with the gas pressures corresponding to the gross and net diastatic activity figures, the coefficients being considerably higher than those for the corresponding relationship between the maltose values and dextrin.

Variables	Coefficient
$D \times G_1$	0.341
$D \times G_2$	0.788 <sup>++</sup>
$D \times G_3$	0.622 <sup>++</sup>
$D \times G_4$	0.763 <sup>++</sup>

Variables	Coefficient
$D \times G_g$	0.822 <sup>++</sup>
$D \times G_n$	0.836 <sup>++</sup>

*Estimation of Gas Production.* Various combinations of the analytical data were used in an attempt to find the most satisfactory set for determining the gas formation of the natural flours in different time intervals. The best estimate of gas formed in the first three hours was achieved by using the factors percent of dextrin (D), total sugars originally present in the flour (T), and susceptible starch (S) as measured by the staining technique, the multiple correlation coefficient being 0.891, although the other combinations tried gave values which were almost as satisfactory. The best estimates for the two periods, the first five hours ( $G_3$ ) and from the end of the third to the end of the seventh hour ( $G_2$ ), were achieved by using the net diastatic activity figure ( $A_n$ ) and the sugars originally present in the flours (T), the values for the over-all correlations being 0.965 and 0.935 respectively. Susceptible starch ( $M_B$ ), the logarithm of the dextrin figure ( $\log D$ ), and the sugars originally present (T) gave the highest multiple correlation coefficient (0.980) for the total gas formation.

For the artificial flours the least satisfactory estimates were those for the first three hours ( $G_1'$ ), the best over-all correlation obtained

in this case (0.793) being that relating the net diastatic activity ( $A_n'$ ), the sugars originally present (T), and  $G_1'$ . For the other time intervals the use of the sugars originally present (T) combined with either the gross ( $A_g'$ ) or net ( $A_n'$ ) diastatic activity gave multiple correlation coefficients of the same order as those obtained for the natural flours using the same variants.

TABLE VII  
STATISTICS RELATING TO PREDICTION OF GAS PRODUCTION

Natural flours					Artificial flours				
Independent variables	Dependent variable				Independent variables	Dependent variable			
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>		G <sub>1</sub> '	G <sub>2</sub> '	G <sub>3</sub> '	G <sub>4</sub> '
CORRELATION COEFFICIENTS									
M <sub>β</sub> , D, T	0.873**	0.915**	0.941**	0.938**	M <sub>β</sub> , T	0.780**	0.932**	0.953**	0.967**
M <sub>β</sub> , T after adjustment for D	0.856**	0.755**	0.903**	0.844**					
A <sub>n</sub> , T	0.875**	0.935**	0.965**	0.931**					
A <sub>g</sub> , T	0.852**	0.886**	0.917**	0.850**					
G <sub>β</sub> , G <sub>n</sub> , G <sub>T</sub>	0.904**	0.951**	0.994**	0.999**	G <sub>β</sub> , T	0.793**	0.942**	0.983**	0.955**
					A <sub>g</sub> ', T	0.788**	0.980**	0.975**	0.987**
					G <sub>β</sub> , G <sub>n</sub> ', G <sub>T</sub>	0.866**	0.976**	0.988**	0.999**
STANDARD ERROR OF ESTIMATE									
M <sub>β</sub> , D, T	20.5	28.4	26.7	92.0	M <sub>β</sub> , T	18.4	30.9	24.9	41.0
A <sub>n</sub> , T	19.7	24.1	20.0	93.7	A <sub>n</sub> ', T	17.9	28.5	15.2	47.3
A <sub>g</sub> , T	21.3	31.5	30.6	135.1	A <sub>g</sub> ', T	18.1	16.9	18.1	25.6
G <sub>β</sub> , G <sub>n</sub> , G <sub>T</sub>	18.0	21.7	8.6	0	G <sub>β</sub> ', G <sub>n</sub> ', G <sub>T</sub>	15.2	19.3	13.1	0
PER CENT OF VARIANCE NOT ACCOUNTED FOR BY REGRESSION									
M <sub>β</sub> , D, T	28.8	19.8	13.8	14.6	M <sub>β</sub> , T	44.4	14.9	10.5	7.5
M <sub>β</sub> , T after adjustment for D	30.5	49.2	21.2	32.8					
A <sub>n</sub> , T	26.6	14.3	7.7	15.2					
A <sub>g</sub> , T	31.1	24.4	18.1	31.5					
G <sub>β</sub> , G <sub>n</sub> , G <sub>T</sub>	22.2	11.6	1.4	0	A <sub>n</sub> ', T	42.1	12.7	3.9	9.9
					A <sub>g</sub> ', T	43.0	4.5	5.5	2.9
					G <sub>β</sub> , G <sub>n</sub> ', G <sub>T</sub>	30.3	5.8	2.9	0

A measure of the differences between actual and estimated gas production in the various time intervals is given by the standard error of estimate (Table VII). The estimation of gas pressure developed during the first three hours is poor, regardless of the variables used for prediction. The most satisfactory predictions of  $G_2$  and  $G_3$  are naturally based on gas pressure values ( $G\beta$ ,  $G_n$ ,  $G_T$ ), while the best estimates for these two time intervals based on sugar values depend on the net diastatic activity figure ( $A_n$ ) and the sugar originally present (T). The gross diastatic activity figure is in each case less satisfactory than the net diastatic activity.

The error of estimate for the artificial flours, expressed as percentage of variance not accounted for, is only slightly less than for the

TABLE VIII

STATISTICS RELATING TO PREDICTION OF GAS PRODUCTION CORRESPONDING TO GROSS AND NET DIASTATIC ACTIVITY

Statistics	Natural flours			Artificial flours		
	Independent variables	Dep. variable		Independent variables	Dep. variable	
		G <sub>g</sub>	G <sub>n</sub>		G <sub>g</sub> '	G <sub>n</sub> '
Corr. coeff.	M <sub>β</sub> , D	0.891* <sup>1</sup>	0.857**			
Corr. coeff. after adjusting for D	M <sub>β</sub>	0.607* <sup>1</sup>	0.338	M <sub>β</sub>	0.955**	0.911**
Error of est.	M <sub>β</sub> , D	108.3	108.3	M <sub>β</sub>	39.3	35.7
Variance not accounted for, %	M <sub>β</sub> , D	23.3	30.1			
Ditto, after adjusting for D	M <sub>β</sub>	67.4	94.5	M <sub>β</sub>	9.3	18.1

natural flours. However, such an observation is open to question since in one series the amylase level is variable and in the other it is constant. In order that a theoretically more satisfactory comparison can be made between the two series of flours, it can be carried out after allowance has been made for the variance due to alpha-amylase (D). This correction has been made experimentally for the artificial series and mathematically for the natural series of flours. With the exception of the first three hours, the proportion of the total variance unaccounted for in the natural flours greatly exceeds that unaccounted for in the artificial series or, expressing it differently, the importance of susceptible starch in determining the magnitude of the standard error is much greater in the case of the artificial flours. In the case of the natural flours much more of the original variance has evidently to be accounted for in some other way, such as by an unknown variable.

When the variants considered are M<sub>β</sub>, D, and T, the actual standard error, expressed as mm. of pressure per 10 g. of flour, is approximately the same for the natural and artificial flours for all intervals except the total gas (G<sub>4</sub>, G<sub>4</sub>'). However, for the variants T and A<sub>g</sub> or A<sub>n</sub>, the standard error of the natural flours is greater than that for the artificial flours in all periods except one.

The magnitude of the residual variance which is unaccounted for in either flour series could not be explained by what is known of the standard deviation of the variants concerned. Thus there may also be an unknown variant in the starch substrate that survives the treatment given to destroy the amylases and is common to both the natural and artificial flour series. If there were a difference in susceptibility of the undamaged starch to a raw starch-splitting amylase, it would

be expected to be a contributing factor in this portion of the standard error.

When the standard error of estimate of the gas pressures corresponding to the gross and net diastatic activity figures of the natural flours is compared with that for the artificial series (Table VIII), it is evident that these exhibit the same peculiarities as does the total gas ( $G_4$ ,  $G_4'$ ), i.e., a better proportional correction of the error of estimate is effected by these variables for the artificial flours and the final error is numerically lower than for the natural flours. The standard error of estimate of the net and gross diastatic activity figures for the same series of flours (Dadswell and Gardner, 1947) showed a different trend,

TABLE IX  
ESTIMATION OF GAS FORMED IN DIFFERENT PERIODS IN NATURAL FLOURS

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$G_1$	
0.2094 $A_g$	+ 0.5249 T + 51.96
0.02923 $M_\beta$	+ 6.208 D + 0.6039 T + 23.27
0.4395 $A_n$	+ 0.5543 T + 47.22
0.05342 $G_\beta$	+ 0.08998 $G_n$ + 0.6081 $G_T$ + 74.48
$G_2$	
1.072 $A_g$	- 0.01989 T - 44.64
0.8381 $M_\beta$	+ 21.61 D + 0.1872 T - 137.70
1.918 $A_n$	+ 0.1784 T - 60.95
0.5305 $G_\beta$	+ 0.2263 $G_n$ - 0.04822 $G_T$ - 20.06
$G_3$	
0.8924 $A_g$	+ 0.6003 T + 3.394
0.5465 $M_\beta$	+ 19.84 D + 0.8163 T - 83.73
1.677 $A_n$	+ 0.7538 T - 12.12
0.4155 $G_\beta$	+ 0.2235 $G_n$ + 0.7297 $G_T$ + 37.15
$G_4$	
3.263 $A_g$	+ 1.161 T - 170.5
1.400 $M_\beta$	+ 85.80 D + 2.120 T - 560.3
6.333 $A_n$	+ 1.694 T - 231.93

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the percentage of reduction effected by consideration of susceptible starch being the same for the gross diastatic activity in the two groups of flours, but greater in the case of the artificial flours for the net diastatic activity. The actual reduction is much greater for the latter series, the final values being such that the natural flours give much lower errors of estimate than those of the artificial series.

The combination  $A_g$ , T, or  $A_n$ , T each contain one variant involving the interaction between susceptible starch, whether in the form of damaged starch or of undamaged starch of varying susceptibility, and any raw starch-splitting amylases. Under conditions of varying alpha-amylase content the gross diastatic activity ( $A_g$ ), although intimately related to susceptible starch and the net diastatic activity, is less satisfactory when judged on the basis of variance unaccounted for

than are those two factors when considered as separate entities in the estimation of gas formation. Under conditions of uniform kind and concentration of amylase, however, the gross diastatic activity gives a slightly better estimate of potential gas formation in the later periods than does the net diastatic activity figure, especially between the end of the third hour and the end of the seventh hour.

The equations relating the potential gas production in each time interval to the different variables used are shown in Table IX for the natural flours and in Table X for the artificial flours. These equations were obtained as customary by use of regression coefficients.

TABLE X  
ESTIMATION OF GAS FORMED IN DIFFERENT PERIODS IN ARTIFICIAL FLOURS

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$G_1'$
$0.02292 M_\beta + 0.4219 T + 218.0$
$0.02883 A_g' + 0.3946 T + 214.8$
$0.04774 A_n' + 0.3864 T + 212.9$
$-0.2736 G_\beta + 0.2683 G_n' + 0.3605 G_T + 189.66$
$G_2'$
$2.3645 M_\beta + 0.2332 T - 38.88$
$0.6511 A_g' + 0.1660 T - 103.7$
$0.8511 A_n' + 0.1826 T - 123.15$
$1.100 G_\beta + 0.2224 G_n' + 0.4108 G_T - 94.46$
$G_3'$
$1.6802 M_\beta + 0.6348 T + 177.88$
$0.5078 A_g' + 0.5335 T + 126.8$
$0.6840 A_n' + 0.5298 T + 108.3$
$0.4643 G_\beta + 0.4616 G_n' + 0.7171 G_T + 100.7$
$G_4'$
$3.762 M_\beta + 1.050 T + 143.86$
$1.0447 A_g' + 0.9328 T + 39.80$
$1.369 A_n' + 0.9559 T + 8.252$

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A comparison of the estimated and actual gas production has been made graphically in Figures 1 and 2. The natural flour samples have been arranged in order of increasing gas production. The gas actually produced by the artificial flours and the calculated values for both the natural and artificial flours have been arranged in the same order. It is at once obvious that the agreement between the actual and estimated gas production figures for the artificial flours is much better than it is for the natural flours. It is also evident that the estimated gas production of the natural flours is highly correlated with the actual and estimated gas production of the artificial flours.

The marked disagreement between the actual and estimated values in some samples may be due to one or more variants which must still be accounted for. A better agreement is observed when the estimation is based on the gas pressures  $G_\beta$ ,  $G_n$ , and  $G_T$ .  $M_\beta$  and  $T$  are

closely correlated with  $G_\beta$  and  $G_T$ . If a better correlation could be obtained between  $A_n$  and  $G_n$ , as in the case of  $A_n'$  and  $G_n'$ , a better estimate of potential gas production would be possible.

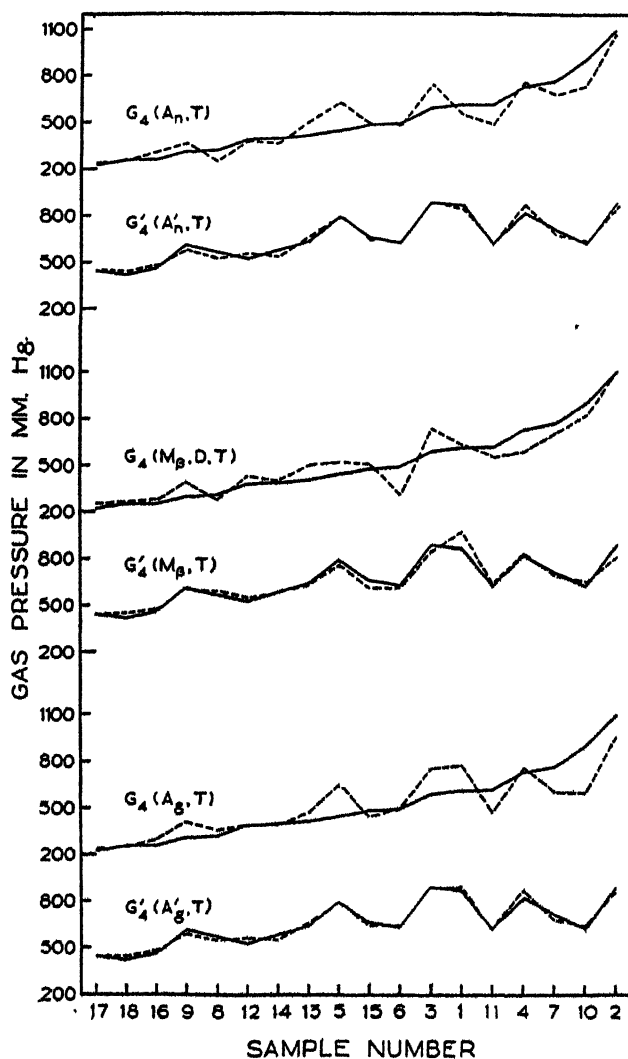


Fig. 1. Actual (—) and estimated (-----) values of the total gas produced in natural flours ( $G_4$ ) and in artificial flours ( $G_4'$ ), based on equations in Tables IX and X.

Figure 3 was prepared from the information available regarding the effect of variations in susceptible starch, alpha-amylase, and sugars originally present on the gas produced in relation to time. The maximum and minimum values used for each variant are those for the



range covered by the samples studied in this experiment. Each variant has been treated separately and in various combinations with the others in order to show the effect on gas production of this range from

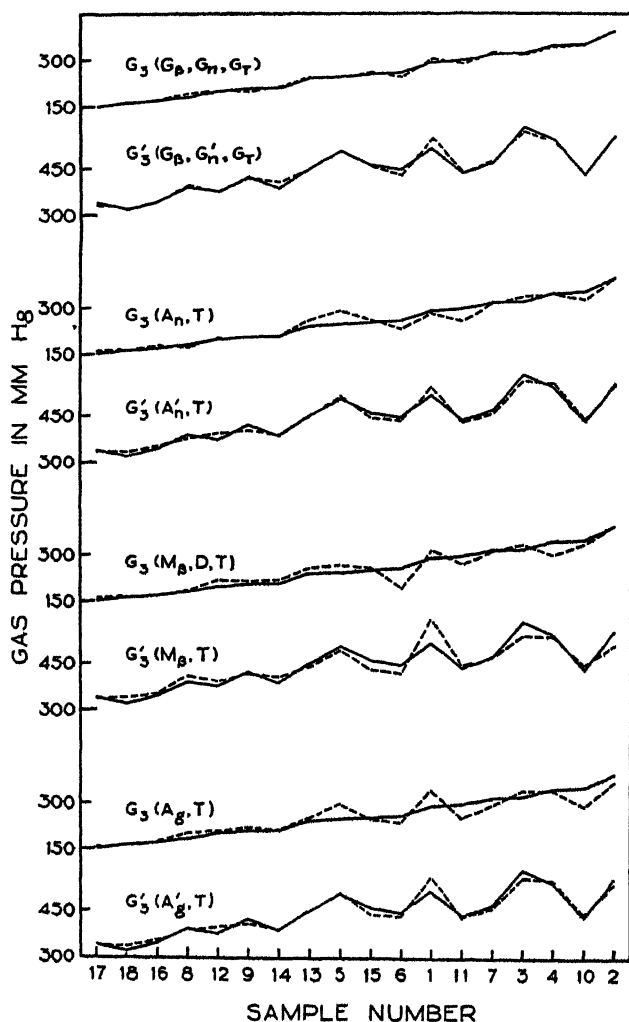


Fig. 2. Actual (—) and estimated (-----) values of the gas produced during the first five hours in natural flours ( $G_i$ ) and in artificial flours ( $G'_i$ ), based on equations in Tables IX and X.

minimum to maximum values. Variation in  $M_p$  has little effect on gas production in the first three hours but has its greatest effect in the fourth hour, after which it gradually declines in importance.  $D$  has some effect during the first three hours, is at a maximum in the fourth hour, and maintains a substantial but slowly declining gas production

subsequently.  $T$  exerts its maximum influence in the first three hours, after which its effects diminish rapidly. Maximum values of  $T$  and  $D$  together give the highest as well as the most sustained gas production, while maximum values in  $M_B$  and  $T$  result in a less prolonged gas production.

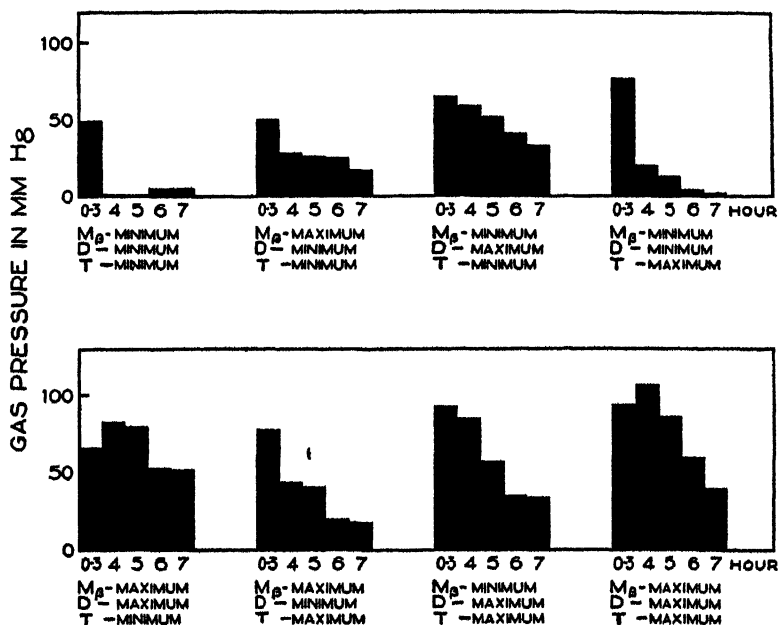


Fig. 3. Effect of variation in susceptible starch ( $M_B$ ), alpha-amylase ( $D$ ), and sugar, originally present ( $T$ ) on hourly gas production, as based on the equations in Table IX. (The values designated 0-3 hour represent the average gas production per hour during the first three hours.)

### Summary

Flours prepared from six varieties of wheat, each grown at three places, have been studied with reference to their gas production.

Variety and place of growth were found to be related to the sugars originally present in these flours and to the gas formed in doughs made from an artificial series of flours having a constant amylase content. The gas formed by the natural flours varies in its relation to variety and place depending on the interval of gas production considered.

There is a high correlation between both sugars originally present and those formed as a result of amylase action and the gas formed from them under conditions of constant or variable amylase concentration.

The gas produced in different time intervals is significantly correlated with the gross and net diastatic activity figures in the case of both the natural and artificial flours.

Estimations of potential gas formation in different gassing periods under conditions of variable alpha-amylase content were more satisfactory when based on the net diastatic activity figure and the sugars originally present than when based on the latter factor and the gross diastatic activity. When constant amounts of alpha- and beta-amylase were present as in artificial flours, the two methods were equally satisfactory in the early gassing periods, but for later periods the net diastatic activity and total sugars gave better results.

The sugars originally present in the flour are important in the early stages of gas formation but have practically no significance later. A variation in the amount of susceptible starch becomes apparent after the end of the third hour, showing that this is partly responsible for maintaining gas formation in the later stages of fermentation. The effect of alpha-amylase is apparent during the first three hours, but this enzyme exerts its maximum activity in the fourth hour, after which its effect gradually declines; but it also is partly responsible for maintaining gas formation in the later stages of fermentation.

There is evidence that, in addition to the factors generally recognized as being responsible for variations in the gas production of natural flours, such as variable sugar and alpha-amylase content, there are one or more additional factors; one is the amount of susceptible starch, while the character of the others has still to be determined.

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# DOUGH OXIDATION AND MIXING STUDIES. VII. THE ROLE OF OXYGEN IN DOUGH MIXING

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The research reported here is concerned with the effects of molecular or gaseous oxygen in dough mixing. In a previous investigation on this subject, Baker and Mize (1937) obtained improved bread quality upon mixing doughs in oxygen for normal intervals; but their work tended to emphasize the harmful effects of overmixing in oxygen on dough and bread quality. Later, Freilich and Frey (1939, 1941) reported the following effects when doughs were mixed in oxygen in the Hobart-Swanson mixer for normal time intervals: (a) the protease activity of papain was inhibited in the patent flour to which it was added, (b) the detrimental effects of reducing matter added to patent flour were minimized, and (c) the quality of bread made from low grade flour was improved. Differences in the condition of the doughs after fermentation and in loaf volume and bread quality were the criteria used for noting these effects. The present research evaluates the effects of oxygen in dough mixing in terms of dough development and bread quality.

## Materials and Methods

Patent or bakers grade flours were used, and dough development was followed by means of farinograph curves. Doughs were mixed and bread baked both by the straight and sponge procedures, employing the following formula:

Flour --100% (300' g), water (variable) 62-64%, yeast 2%, sucrose --5%, salt --2%, shortening 3%.

In the experiments with papain, Figures 6 and 7, 3% milk powder was used in addition to the other ingredients.

The Brabender Farinograph was used in most of the mixing experiments. It was fitted with a cover having a rubber gasket which also fitted the rim of the mixing bowl. The cover was provided with two openings, one for admitting the gas used and the other for connection with a manometer. Mixing was done under slight positive pressure (about 10 mm of Hg).

The straight doughs were mixed in the farinograph (unless otherwise noted) for sufficient time to determine whether a peak occurred in the farinograph curve as dough development proceeded. This usually occurred between 12 and 18 minutes. After about two hours'

fermentation at 30°C, when a dough volume of 1180 ml was attained, 480-g portions were rounded, molded by machine, proofed at 40°C to the top of the pan, and baked for 30 minutes at 215°C. In some experiments, the dough was remixed after fermentation and this is so indicated in each instance.

The sponges (60% flour, 37% water, and the yeast)<sup>1</sup> were mixed in the Hobart-Swanson mixer for two minutes, fermented for three and one-half hours at 30°C, and then mixed with the dough ingredients in the farinograph long enough (approximately 15 minutes) to show whether or not a peak in dough development was being indicated. After a 20-minute dough time, 480-g portions of the doughs were rounded, molded, proofed, and baked in the same manner as were the straight doughs.

### Effects of Oxygen on Dough Development

Good development is a condition attained by doughs during mixing, and is characterized by maximum dough consistency or firmness and good elasticity. These properties are correlated with optimum bread quality factors, such as loaf volume, texture, and grain. The farinograph curve gives a good index of the course of dough development during mixing, and mixing time, consistency, and elasticity of the dough may be evaluated from the curve.

The effects of mixing straight doughs in oxygen and in nitrogen are shown in Figure 1. Oxygen had a very profound effect on the dough. The curve for a straight dough mixed in oxygen before fermentation shows good consistency and elasticity and goes through a definite peak. But the curve for a straight dough mixed in nitrogen before fermentation is flat, showing practically no development or increase in firmness during mixing. These curves indicate the fundamental importance of oxygen as a factor in proper dough development; such development is evidently unobtainable in the absence of oxygen.

It is also evident that oxygen is most effective when incorporated into the dough during the original mixing. The dough which was mixed in nitrogen, then remixed in oxygen after fermentation, does show development, but it required 30 minutes to attain that stage, as against 12 minutes for the dough mixed in oxygen originally. The dough which was remixed in nitrogen after fermentation, following an original mixing in nitrogen, showed little development even after 30 minutes of mixing, again indicating the essential need of oxygen in dough development.

That satisfactory dough development can be achieved by incorporation of oxygen after fermentation is illustrated in Figure 2. All

<sup>1</sup> The amounts used in the sponge were 180 g flour, 110 ml water, 6 g yeast (300 g 100%).

of the doughs from which these loaves were baked were mixed in nitrogen for four minutes in the Hobart-Swanson mixer (this mixer develops doughs much more rapidly than the farinograph). After a normal fermentation period of about two hours, until a dough volume

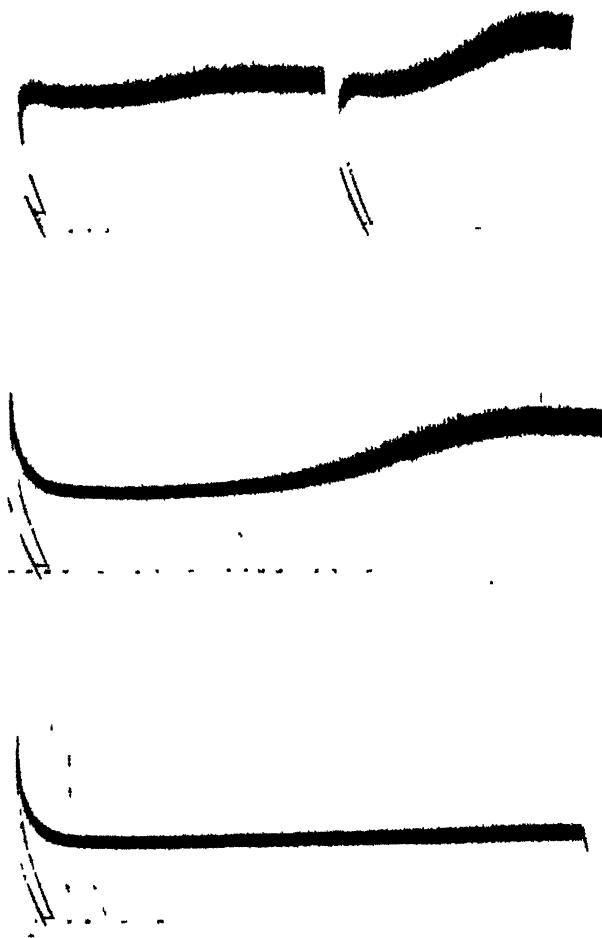


Fig. 1. Farinograph curves of straight doughs mixed in nitrogen and oxygen.  
Top, left—Dough mixed in nitrogen before fermentation.  
Top, right—Dough mixed in oxygen before fermentation.  
Center—Dough mixed in oxygen after fermentation, following original mixing in nitrogen.  
Bottom—Dough mixed in nitrogen after fermentation, following original mixing in nitrogen.

of 1180 ml was reached, doughs No. 1, 2, and 3 were remixed in the same mixer in air for one, five, and ten minutes respectively, and doughs No 4, 5, and 6 were remixed in the same mixer in nitrogen, also for one, five, and ten minutes. Loaves 2 and 3 were of normal volume and texture, indicating that a 5-10 minute remix in air provided sufficient oxygen for proper dough development. But all the loaves baked from doughs mixed in nitrogen showed inferior volume and texture due to lack of dough development during remixing in the absence of oxygen.



Fig 2 Bread baked from straight doughs which were mixed in nitrogen, fermented, and remixed in air or nitrogen

Dough No 1—Remixed in air for 1 minute  
 Dough No 2—Remixed in air for 5 minutes  
 Dough No 3—Remixed in air for 10 minutes  
 Dough No 4—Remixed in nitrogen for 1 minute  
 Dough No 5—Remixed in nitrogen for 5 minutes  
 Dough No 6—Remixed in nitrogen for 10 minutes

Figure 3 illustrates the effects of mixing fermented sponges with the dough ingredients in air, nitrogen, and oxygen. The oxygen curve shows much higher consistency<sup>2</sup> and shorter mixing time than the nitrogen curve, the air curve shows similar effects of lesser magnitude. Loaf quality showed the same order of improvement, from mixing in nitrogen to mixing in oxygen. Thus, it is concluded that oxygen is essential in the mixing of sponge doughs, as well as in the mixing of straight doughs, if satisfactory dough development and loaf quality are to be obtained.

### Effects of Oxygen in Doughs Made with Different Bread Flours

The farinograph curves in Figures 4 and 5 illustrate the progressive effects of increasing concentrations of oxygen in the mixing of doughs made from three samples of Northwestern flour and three samples of Kansas flour. These were all patent flours, 72% extraction. They were used in straight doughs which were mixed in nitrogen, air, and oxygen.

<sup>2</sup> "Consistency" is the term which appears on the graph paper used in the farinograph to designate values on the vertical axis from 0 to 1000, 'higher consistency' refers to these numerical values



The curves for doughs mixed in nitrogen show little change from beginning to end of mixing, indicating a lack of dough development. Here the mixing times are difficult to estimate because the curves do not go through a definite peak.

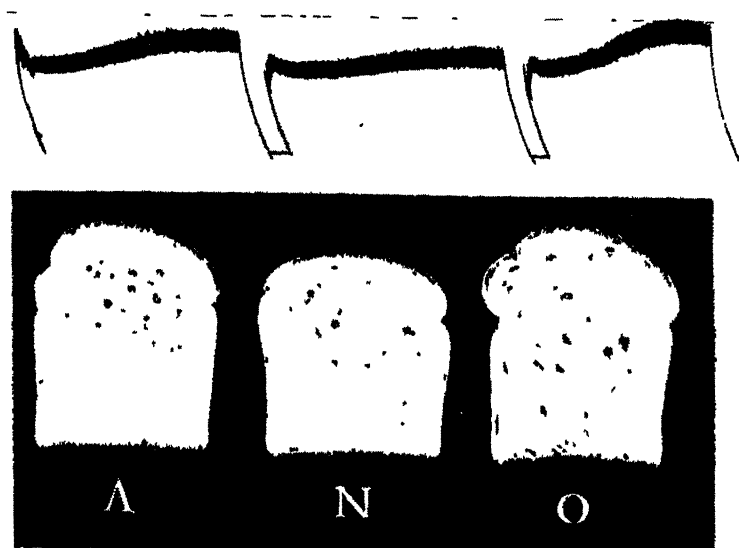


Fig. 3. Farinograph curves and bread baked from sponge doughs. Sponges all mixed in air before fermentation.

Fermented sponges mixed with dough ingredients in:

A = air  
N = nitrogen  
O = oxygen

The curves for the doughs mixed in air and in oxygen show progressively better dough development, as evidenced by higher consistency and greater elasticity (indicated by increased width at the peaks of the curves). Oxygen has a tendency to shorten the mixing time required for maximum dough development.

Mixing in nitrogen always resulted in bread of inferior volume and quality (Figures 4 and 5). The bread produced from the doughs mixed in air was much superior to the corresponding nitrogen loaves in all respects. Mixing in oxygen produced additional improvement in volume and quality, though not to the extent indicated by air over

nitrogen. Two of the six flours showed no improvement due to oxygen as compared to air. It was therefore indicated that, though the oxygen requirements of different flours may vary in degree, the total lack of oxygen in mixing prevents satisfactory dough development, with a consequent inferiority in the quality of the resulting bread.

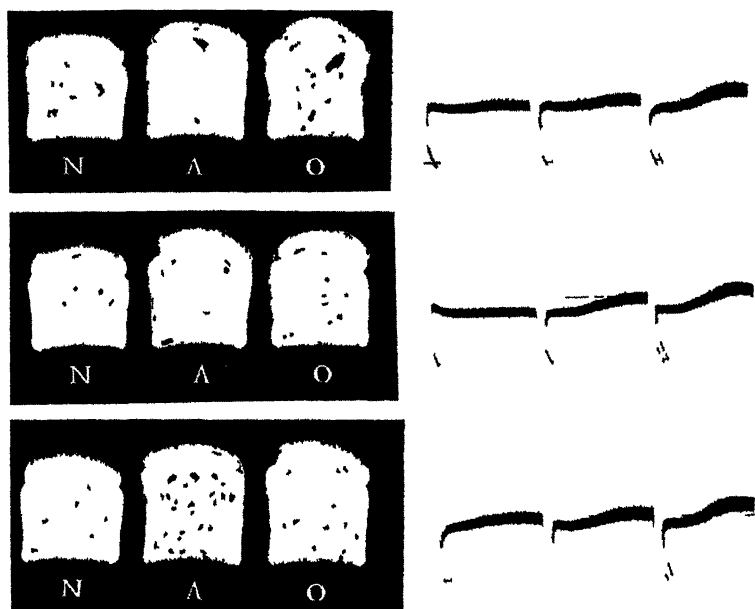


Fig 4 Farinograph curves and bread baked from straight doughs made from different flours. Top row—Northwestern special patent, middle row—Northwestern bakers flour, bottom row—Northwestern patent flour. Before fermentation, doughs were mixed in N = nitrogen, A = air, O = oxygen. See Table I for additional data.

The farinograph and baking data for these flours are given in Table I. From the dough times, it is seen that oxygen retarded fermentation in its initial stages, but that the rate of gas production was back to normal or somewhat faster during the proofing stage. This initial effect of oxygen may be explained on the basis of aerobic respiration. Yeast metabolism may be either anaerobic (fermentation) or aerobic (respiration). The presence of oxygen favors respiration, so that fermentation is retarded in the dough until the oxygen is used up. This effect in sugar solutions has long been known. For a discussion of the effect, see Werkman (1946).

#### Effects of Oxygen in Doughs Containing Added Protease

The beneficial effects of oxygen on dough development have been shown to be immediate, that is, they become apparent during the first

few minutes of dough mixing. The harmful effects of protease activity, on the other hand, are known to be delayed, particularly when protease is present at low concentration. In patent or bakers grade flours, the protease content is so minute as to be of little practical significance. It would therefore seem that the effects of oxygen during dough mixing do not involve protease activity, but that the oxygen probably acts on the gluten complex.

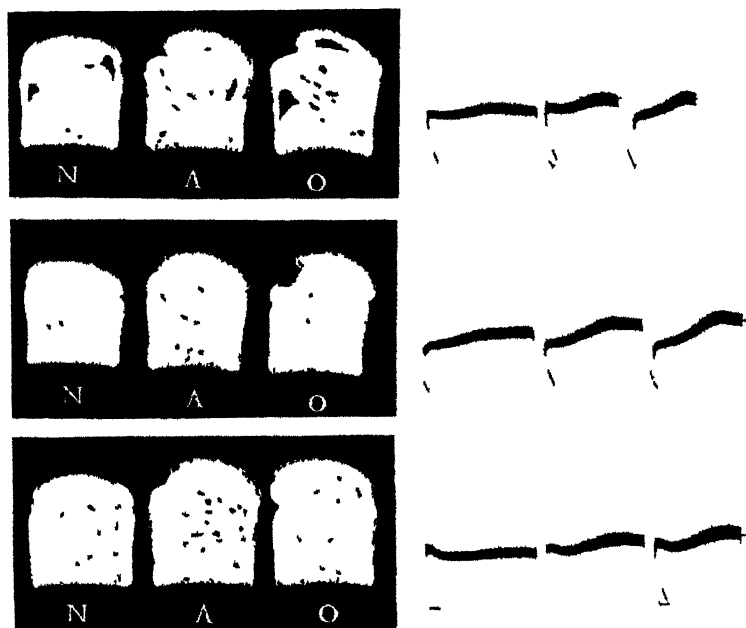


Fig 5 Farinograph curves and bread baked from straight doughs made from different flours. Top row—Kansas hard winter patent, middle row—Kansas flour, bottom row—Kansas bakers flour. Before fermentation, doughs were mixed in N = nitrogen, A = air, O = oxygen. See Table I for additional data.

To substantiate the theory that the oxygen effect during mixing is independent of protease activity of the papain type, which manifests itself only during subsequent processing of the dough, straight doughs with and without added papain were mixed in nitrogen and in oxygen in the farinograph. The curves showed the usual effects of mixing in oxygen, that is, increased consistency and elasticity, and shorter mixing time, but no effects of comparable magnitude were indicated due to the presence of papain during mixing (Figure 6). After several hours of fermentation, however, the effects of papain became apparent, as evidenced by inferior bread quality in loaves 2 and 3, made from doughs mixed in nitrogen. Papain produced no harmful effects in the doughs mixed in oxygen (loaves 5 and 6).

TABLE I  
 FARINOGRAPH AND BAKING DATA FOR DIFFERENT FLOURS  
 (Straight doughs, mixed in nitrogen, air, and oxygen)

Type of flour	Gas used in mixing	Mixing time	Consistency	Dough time	Pan proof time	Loaf volume	Bread quality
		<i>Min.</i>	<i>B.U.<sup>2</sup></i>	<i>Min.</i>	<i>Min.</i>	<i>ml</i>	
Northwestern special patent	Nitrogen	12.0 <sup>1</sup>	450	113	52	1700	Dense; below normal.
Northwestern special patent	Air	13.0	500	113	52	1880	Close to normal; slightly dense.
Northwestern special patent	Oxygen	13.0	570	131	51	1920	Normal, though slightly open.
Northwestern bakers flour	Nitrogen	13.0 <sup>1</sup>	390	112	56	1590	Dense; slightly coarse, below normal.
Northwestern bakers flour	Air	13.5	490	110	56	1830	Normal.
Northwestern bakers flour	Oxygen	12.5	550	124	55	1830	Normal; slightly over-oxidized.
Northwestern patent	Nitrogen	12.5 <sup>1</sup>	490	105	50	1640	Dense; below normal.
Northwestern patent	Air	13.0	520	108	50	1830	Close to normal.
Northwestern patent	Oxygen	12.0	590	122	52	1910	Normal.
Kansas hard winter patent	Nitrogen	9.5 <sup>1</sup>	420	105	53	1680	Slightly dense; below normal.
Kansas hard winter patent	Air	8.5	490	106	59	1880	Close to normal.
Kansas hard winter patent	Oxygen	8.75	510	119	56	1880	Close to normal.
Kansas bakers flour	Nitrogen	12.5 <sup>1</sup>	400	111	55	1660	Dense; below normal.
Kansas bakers flour	Air	11.0	480	114	54	1940	Normal.
Kansas bakers flour	Oxygen	10.5	520	126	52	1910	Slightly finer grain than in air-mixed loaf.
Kansas flour	Nitrogen	13.0 <sup>1</sup>	420	106	56	1740	Dense; below normal.
Kansas flour	Air	10.25	480	110	56	1910	Normal.
Kansas flour	Oxygen	9.75	520	126	56	1970	Normal; rough break.

<sup>1</sup> Mixing time indefinite in nitrogen.

<sup>2</sup> B. U. = Brabender Units.

In another experiment, doughs with and without added papain were mixed in the farinograph in nitrogen and in oxygen, then remixed in the farinograph in air, after several hours of fermentation. Following the remixing, the doughs were allowed to rest for 20 minutes, and then were rounded, molded, proofed, and baked as usual. The farinograph curves in Figure 7 showed significant differences due to the action of papain, in contrast to the curves in Figure 6. The papain produced more rapid dough development during remixing, as indi-

cated by higher consistency in the doughs originally mixed in nitrogen, and shorter mixing times in all doughs. For the doughs originally mixed in nitrogen, bread quality was related to dough development; the papain doughs showed better development during remixing and produced better bread than the control. The papain doughs (No. 5 and 6) originally mixed in oxygen produced satisfactory bread, comparable to the control bread (No. 4), and better than bread from doughs mixed in nitrogen (No. 1-3).

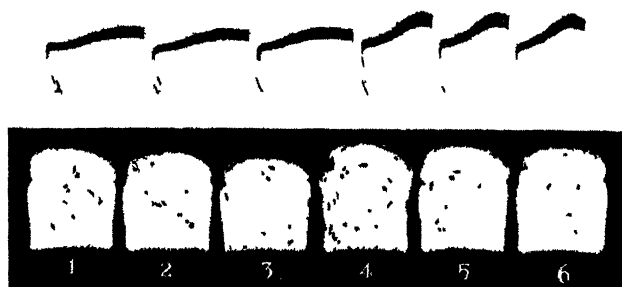


Fig. 6 Effect of added protease (papain) upon farinograph curves and bread baked from straight doughs mixed in nitrogen or oxygen.

Doughs mixed in nitrogen before fermentation:

Dough No. 1—No added papain.

Dough No. 2—5 mg papain.

Dough No. 3—10 mg papain.

Doughs mixed in oxygen before fermentation.

Dough No. 4—No added papain.

Dough No. 5—5 mg papain.

Dough No. 6—10 mg papain.

This phase of the investigation of the effects of oxygen in mixing doughs of high and low protease content may be summarized as follows:

1. In patent or bakers grade flours, in which the protease content is known to be practically insignificant, the beneficial effects of oxygen upon dough development during mixing, as shown in farinograph curves, are independent of any protease that may be present.

2. In doughs made from flours containing added protease, the effects of oxygen during dough mixing are also independent of protease activity, as shown by the farinograph curves. However, the harmful effects of protease in these doughs become apparent during subsequent processing after fermentation, as evidenced by inferior bread quality. These harmful effects of the protease are minimized and bread quality is normal when oxygen is present in sufficient quantities during mixing or during subsequent processing.

These results, therefore, indicate that the effects of oxygen during dough mixing are independent of any protease of the papain type that may be present in the flour. Oxygen produced effects that were immediately apparent, whereas the effects of added papain, both in the dough and in the resulting bread, became evident only after several hours of dough fermentation.

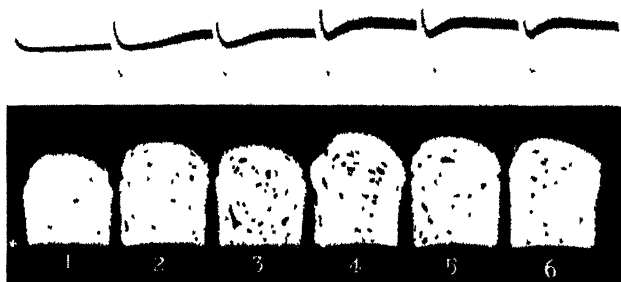


Fig. 7. Effect of added protease (papain) upon farinograph curves and bread baked from straight doughs mixed in nitrogen or oxygen, fermented and then remixed in air.

Doughs mixed in nitrogen before fermentation and remixed in air:

Dough No. 1—No added papain.

Dough No. 2—5 mg papain.

Dough No. 3—10 mg papain.

Doughs mixed in oxygen before fermentation and remixed in air:

Dough No. 4—No added papain.

Dough No. 5—5 mg papain.

Dough No. 6—10 mg papain.

### Mechanism Involved in the Effects of Oxygen

The effects of oxygen in dough mixing were so pronounced that fundamental changes, possibly chemical in nature, were indicated. Since these changes were effected by molecular oxygen it appeared probable that an enzymic oxidation mechanism was involved. If this were so, it should be possible either to inhibit the reaction by inhibiting or inactivating the enzymes, or to obtain more pronounced effects by using a higher concentration of oxidizing enzyme. Both of these possibilities were investigated.

Figure 8 shows the effects of 0.5% cuprous chloride, an enzyme inhibitor, on a straight dough mixed in oxygen in the farinograph. The curve is very much like that obtained with a straight dough from the same flour, but mixed in nitrogen and containing no added cuprous chloride (Figure 1), indicating the inhibition of the mechanism involved in the oxygen effect. The enzyme activity of the yeast in the dough was also inhibited by the cuprous chloride.

Indirect evidence tending to confirm the presence of an oxidizing enzyme system was obtained by adding small amounts of quinoa flour to the dough. *Quinnua polylepis*, of the rose family, is a native-grown Bolivian grain widely used as food by the Indians,<sup>1</sup> it is not a wheat variety. Quinoa flour appears to have a much higher concentration of oxidizing enzyme than wheat. Ten percent of the regular flour was replaced by an equal amount of the quinoa flour in doughs which were mixed in air and in nitrogen, as compared to a control dough mixed in air. In another test, 10% quinoa flour was heated in water to 95°C<sup>4</sup>

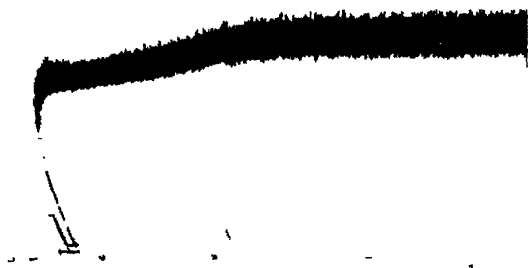


Fig 8 Farinograph curve of a straight dough containing 0.5% of cuprous chloride (flour basis) mixed in oxygen before fermentation. (This curve is comparable to those in Figure 1, the same flour formula and procedure were used for the curves in Figures 1 and 8.)

in order to inactivate the enzyme, then used in a dough which was mixed in air; all the mixing was done in the farinograph. The curves obtained in these tests are shown in Figure 9.

Analysis of Figure 9 indicates that quinoa must have at least several times the concentration of oxidizing enzyme as have American bread flours. The dough containing as little as 10% quinoa flour, when mixed in air, showed a typical oxygen effect and, when mixed in nitrogen, was similar to the control without quinoa flour which was mixed in air.<sup>5</sup> The dough containing 10% quinoa flour that had been heated to inactivate the enzyme was also similar to the control, in that it did

<sup>1</sup> See anonymous article entitled 'Bolivia to use quinoa flour' in *Modern Miller and Bakers News* 73 (51) 16, Dec 21, 1946

<sup>4</sup> See footnote 6

<sup>5</sup> The curve for the quinoa dough that was mixed in nitrogen before fermentation was as good in development as the control curve, this may indicate that the oxygen adsorbed or held mechanically by the flour was sufficient to produce some effect

not show an oxygen effect when mixed in air.<sup>6</sup> Hence, the activity of the system was nullified either by depriving it of the oxygen in the air or by denaturing it with heat.

### Discussion

The role of oxygen in dough mixing is of fundamental importance, but apparently this has not been fully appreciated in the past. Undoubtedly, the good effects attributed to high-speed mixing were actually largely due to the incorporation of increased amounts of oxygen, as compared to low-speed mixing. The essential function of oxygen in dough mixing presupposes a definite mechanism for its utilization in the gaseous or molecular form. The mechanism is apparently enzymic in nature.



Fig. 9. Farinograph curves of straight doughs containing quinoa flour. Reading from left to right the treatments of the doughs were as follows:

- Control (no quinoa flour); dough mixed in air before fermentation.
- 10% quinoa flour; dough mixed in air before fermentation.
- 10% quinoa flour; dough mixed in nitrogen before fermentation.
- 10% quinoa flour which was first heated to 95°C, then used in dough which was mixed in air before fermentation.

According to Jørgensen (1945) the beneficial effects of oxidation in dough are due to protease inhibition. Certain workers have indicated that this theory may be unable to explain the effects of oxidizing agents in dough made from patent or bakers grade flour. Laitinen and Sullivan (1941) and Baker, Parker, and Mize (1942) have placed emphasis on a probable effect on the gluten as an alternative explanation. In mixing, the action of oxygen is immediate and seems to affect the gluten. The reaction is apparently unrelated to protease activity. From this it may be inferred that the effects of oxidizing agents are similar in character. The protease theory may be operative to the

<sup>6</sup> The fact that the curve for the dough containing heated quinoa indicated slightly lower consistency than did the control curve, although paralleling it in shape, may be explained by the additional water which was required by the former dough due to effects produced during heating, such as gelatinization of starch in the quinoa flour; in making this adjustment, too much water was added, producing the lower consistency.



extent that protease is active in the dough, but since the protease content of American bread flours appears to be negligible, the theory must assume a minor role in explaining oxidation effects in doughs made from such flours.

The essential nature of oxygen in dough mixing may lead to practical application in the baking industry, since oxygen produces improvements in dough and bread quality beyond the results obtained by mixing in air.

### Summary

Mixing studies in which both straight and sponge doughs were mixed in the presence and absence of oxygen were made with the aid of the farinograph and general breadmaking procedures.

Oxygen is essential to proper dough development and was most effective when used in the original mixing (before fermentation). When used in mixing after fermentation it was still effective, but the time required for dough development was much longer than when used originally.

Oxygen retarded fermentation in the initial stages, but the fermentation rate was normal in the proofing stage.

The oxygen effects during dough mixing were found to be independent of protease activity.

Some evidence is presented to indicate that oxygen is utilized by means of an enzymic mechanism.

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# EFFECT OF EXCESSIVE METHYL BROMIDE FUMIGATION ON FLOUR<sup>1</sup>

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Control of insect pests in food-processing plants, storehouses, and in stored products by means of fumigation is a very widely accepted practice. Elimination of these pests in the food industries is a matter of considerable importance from an economic as well as a public health standpoint. Successful fumigation practice involves the effective destruction of insects without leaving toxic or undesirable residues in the fumigated product.

Among the many commercial fumigants used, methyl bromide is one of the most recently developed. The use of methyl bromide has increased tremendously within the past few years because it can be easily handled and is extremely toxic to insect life. Several investigations have demonstrated that there are no harmful effects from methyl bromide residues in foodstuffs. However, complaints occasionally do occur regarding the unpleasant odor and taste of products baked from flour fumigated with methyl bromide. It seems apparent that these alterations occur when flour becomes exposed to excessive amounts of fumigant.

The object of this research was to determine the nature of the changes in flour fumigated with methyl bromide and particularly to investigate the factors responsible for the development of undesirable odor and taste. Further, it was desired to study the effect of moisture on the sorption (combined adsorption and absorption) of methyl bromide, as well as any alterations of the physical properties of dough subsequent to fumigation.

To date most of the papers on methyl bromide deal with the techniques of commercial fumigation. However, such topics as the rate of sorption during fumigation, toxicity to laboratory insects, and the effects of bromide residues in foodstuffs on laboratory animals have been investigated. Roehm, Shrader, and Stenger (1942) have determined bromide residues in cereals fumigated with methyl bromide.

Mackie and Carter (1937) presented some of the first evidence in favor of the use of methyl bromide in the fumigation of infested fresh vegetables. They claimed that in using normal dosages the vege-

<sup>1</sup> This paper represents a portion of a thesis presented to the Graduate School of Kansas State College in partial fulfillment of the requirements for the degree of Master of Science.

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<sup>3</sup> Contribution No. 138, Department of Milling Industry.

tables were not damaged. On the other hand, Phillips, Munro, and Allen (1939) found that while methyl bromide is practical in the destruction of insects in harvested apples, under certain conditions the treatment results in both external and internal injury to the fruit. Dudley *et al.* (1940, 1942) found that dried fruits, fresh fruits, and vegetables absorbed minor quantities of the fumigant; but milled grains, cheese, nuts, and nut meats absorbed greater amounts. These authors believed that milled grains sorbed more methyl bromide because of their greater surface area, while the oily and fatty foods sorbed large quantities of the fumigant because of its solubility in fats.

Shepard and Buzicky (1939) reported that baking tests with flour fumigated with methyl bromide at two pounds per 1,000 cubic feet showed no detectable injury. Searls (1943) stated that methyl bromide produced a disagreeable odor on some furs and leathers, but there are no reports in the literature describing the odoriferous reaction of this fumigant with flour.

### Materials and Methods

Preliminary studies were carried out with a bleached and malted commercial flour which had a protein content of 12 0%. Fumigations were performed at 25°C with a laboratory scale, fan-equipped, fumigator having a capacity of 116 liters (4.1 cubic feet). The dosage applied in most of the experimental fumigations was at a very high level of 25 pounds per 1,000 cubic feet for 24 hours, to insure overfumigation. This dosage is 12 to 25 times greater than is used in normal practice. For certain studies methyl chloride at similar dosage levels was also used.

Two years of extensive investigation in this laboratory on the effect of various amounts of methyl bromide applied to flour, on the development of odors in bread or toast made from the flour, demonstrated that several times the normal amount of fumigant are required to produce objectionable odors. When the fumigant is applied at the recommended concentrations of from one to two pounds per 1,000 cubic feet, or in slight excess of this amount, no odor developed after the flour was fumigated and aerated. In this investigation excessively heavy overdosages of methyl bromide were used to produce pronounced effects of overfumigation.

For the study of the relation of methyl bromide sorption to the moisture content of the flour, flours were exposed to different humidities until the desired moisture content was obtained. The samples were then placed in sacks and fumigated. Immediately after fumigation, each sample was thoroughly blended in a sealed mixer. One part was then set aside in a sealed container and the other part was

aerated for seven days at room temperature and then stored in sealed containers. A duplicate sample was allowed to aerate for seven days at room temperature before blending and storing in sealed containers.

Petroleum ether extracts were prepared by extracting flour with several portions of Skellysolve F at room temperature. The extracts were concentrated under vacuum at the same temperature.

The extracted flour was made into a dough using the appropriate amount of water, and fractionated into starch, gluten, and water-soluble material according to the technique described by Finney (1943).

The experiments reported in this work demonstrated that when fumigated flour is treated with a solution of potassium hydroxide or sodium hydroxide in ethyl alcohol, a characteristic, rather unpleasant odor is produced. This test was successfully employed to detect bromide residues due to chemical reactions in fumigated fractions of flour.

The Brabender Farinograph was used to determine the exact water absorption of the samples, to obtain doughs of the same consistency, and to indicate any alteration in the physical dough properties due to fumigation. Arbitrary values were given to the areas under the farinograms by means of the valorimeter as used by Johnson, Shellenberger, and Swanson (1946). The amount of sample employed in every test was corrected to 14% moisture basis.

The optimum dough mixing times were estimated with the Swanson-Working recording dough mixer, using the absorption found with the farinograph. This also permitted additional observations on physical dough properties.

The straight dough baking test method was employed. The formula and baking procedures used were those described by Johnson, Swanson, and Bayfield (1943).

The weight and volume of the loaves were determined immediately after they were taken from the oven. The bread was then placed in sealed cans, and on the following day was scored for grain, texture, exterior appearance, and odor. The odor rating was determined immediately after slicing the loaf. A panel of observers cooperated with this test. Also the original odor observations were followed by similar tests after toasting slices of the bread.

Several methods for determining small quantities of bromine in different materials have been published. In the present work the analytical procedure described by Shrader *et al.* (1942) for total bromide was used.

Gas production and gas retention were determined by means of the apparatus and procedure described by Working and Swanson (1946).

The estimation of the killing power of methyl bromide on the microflora of flour was carried out by the technique recommended by Kent-Jones and Amos (1930) and by Smith and Dawson (1944) for bacterial and fungal counts, respectively.

### Results

*Preliminary Investigations.* Preliminary baking tests with flours fumigated at a dosage of 25 pounds per 1,000 cubic feet gave bread with a sharp, very objectionable odor. Upon cooling the loaves, the odor was not nearly so marked, but became strong again when slices of the bread were toasted. Flour fumigated with heavy dosages of methyl chloride produced baked loaves with a very objectionable odor but different from that given by methyl bromide fumigated flours. This experiment was tried as a side line to determine if a methyl halide other than methyl bromide would develop "off-odor" properties in bread.

In addition to the study of the effects of fumigation on flour it was planned to investigate the effects on the following four flour fractions: the petroleum ether extract, starch, gluten, and water-soluble material. However, early in the studies it was discovered by accident that 5% alcoholic potassium hydroxide (5 g. KOH in 100 ml. of EtOH), when added to fumigated flours, produced an unpleasant odor. The same reagent applied to unfumigated flour produced no odor. The application of this test showed that only the gluten and water-soluble fractions, i.e., the protein-containing fractions of flour, reacted with the fumigant; therefore it appeared unnecessary to consider all four fractions.

However, the petroleum ether extract from flour was at first included because according to Balls *et al.* (1940, 1942) it contained a lipoprotein with a high content of sulfur. It was thought that mercapto groups might perhaps be responsible for the odor obtained in the baked products of fumigated flours. Subsequently, however, a nitroprusside test demonstrated that nonfumigated flour, fumigated flour, fumigated flour treated with alcoholic potassium hydroxide, and an aqueous solution of methionine treated with liquid methyl bromide did not have free sulphydryl groups.

Baking tests were made with nonfumigated flour, nonfumigated extracted flour, fumigated flour, and fumigated extracted flour. Both fumigated flours, extracted and unextracted, yielded products with the same unpleasant odor of approximately similar intensity. The odor was present in the loaves not only shortly after they were taken from the oven, but also on the following day after being stored in sealed cans.

Petroleum ether extracts from nonfumigated and fumigated flours

were treated with 95% ethanol in order to split the lipoprotein present in the extract. The two original extracts and the same extracts after ethanol treatment were fumigated with heavy dosages of methyl bromide. All samples gave a negative odor reaction when treated with alcoholic potassium hydroxide. On the other hand, gliadin and glutenin separated with 70% ethanol from gluten washed from fumigated flours both gave a positive odor test of approximately the same intensity with alcoholic potassium hydroxide.

When gluten was treated with methyl bromide and then added to flour it had a detrimental effect on the baking qualities as evidenced by a reduction in the loaf volume of the bread. This result was obtained by treating washed, dried, and finely ground gluten with liquid methyl bromide. After treatment the gluten was thoroughly aerated at room temperature until no evidence remained of the presence of the volatile fumigant. Two portions of the same flour were baked, one containing a 5% addition of treated gluten, the other an equal quantity of untreated gluten. The results were as follows:

<i>Material</i>	<i>Loaf volume, cc.</i>
Flour (check)	745
Flour plus 5% gluten	857
Flour plus 5% treated gluten	610

When an attempt was made to wash out gluten from flour treated with liquid methyl bromide, a glue-like substance was obtained. Gluten from fumigated flours also shows alteration, but not to such a marked degree.

*Miscellaneous Materials Fumigated with Methyl Bromide.* Twelve different materials were fumigated with methyl bromide at a dosage of 25 pounds per 1,000 cubic feet. These materials were corn flour, rye flour, oat flour, flax flour, barley malt, navy beans, soya beans, sorghum, dried gluten, dried blood fibrin, egg albumin, and gelatin. The alcoholic potassium hydroxide odor test gave positive results for all but gelatin. Thus gelatin was the only material that did not give the characteristic odor with the reagent.

*Influence of Moisture Content on Sorption of Methyl Bromide by Flour.* Flours ranging in moisture content from 9 to 16% were studied for bromide residues after similar methyl bromide fumigation. The extent of bromide sorption after fumigation and aeration at each moisture level is shown in Figure 1. The moisture contents after fumigation agreed within 0.1% of the values before fumigation. Bromide values are expressed in terms of the bromide ion.

The results shown in Figure 1 indicate that at moisture contents higher than 13.7% the sorption of bromide increases with the moisture

content of the flour. The correlation coefficient between the apparent bromide sorbed and moisture content of the flour is  $r = 0.97$  (see Figure 2). At flour moisture levels above 15% the bromide sorbed during fumigation was almost completely retained even after aeration.

Several factors appear to control the sorption of methyl bromide by flour. Gases can diffuse into flour through the free spaces between the flour particles. Water vapor present in the atmosphere of the void spaces hinders diffusion of gases and at the same time it forms a film around the particles. To a great extent, such water films probably control sorption of the fumigant. This would account for the high

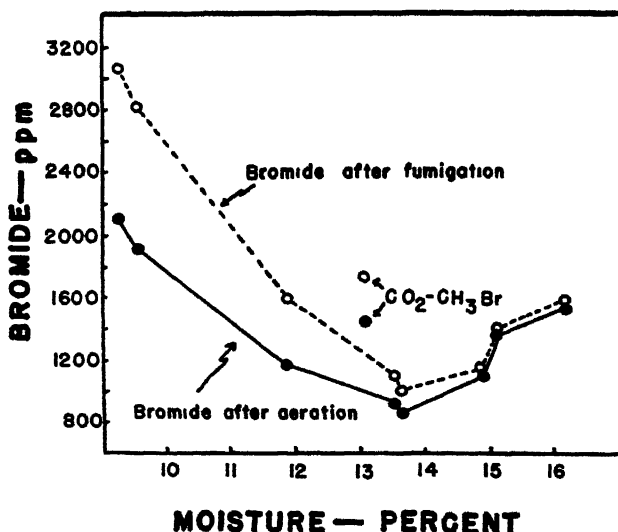


Fig 1 The relationship between moisture content of flour and bromide sorbed after fumigation. The effect of aeration on bromide sorbed is shown. The results of one fumigation with a mixture of carbon dioxide-methyl bromide is indicated by two points at the 13.7% flour moisture level

sorption of methyl bromide, and the comparatively low retention after aeration, in flours containing less than 13.7% moisture. It might be assumed that retention is primarily due to chemical reactions between methyl bromide and proteins and only secondarily to hydrolysis of the fumigant.

When moisture increases, the sorption of the fumigant during fumigation is hindered, but retention is higher due to greater hydrolysis. After a moisture content of about 13.7% is reached, the sorption due to hydrolysis seems to prevail.

Farinograms and mixograms were made of all samples (Figure 4). Table I shows the absorption determined with the farinograph and the values found with the valorimeter from the farinograms. Discussions of the influence of amino acids and carbon dioxide-methyl bromide

fumigated samples appear later. All of the curves for the treated flours had shorter mixing times and more rapid decreases in dough consistency than did the curve of the check flour. The valorimeter readings for the fumigated flours are lower than for the control. It is evident that fumigation caused changes in the flours which altered the physical properties of the doughs. The kind and degree of the alterations seem to be regulated to a great extent by the moisture content of the flour. Thus, samples 7 and 8 (high moisture flours) yielded narrower tracings at the end of the farinograms than did the other samples. They also showed lower consistency at the end of the mixograms than the other fumigated samples.

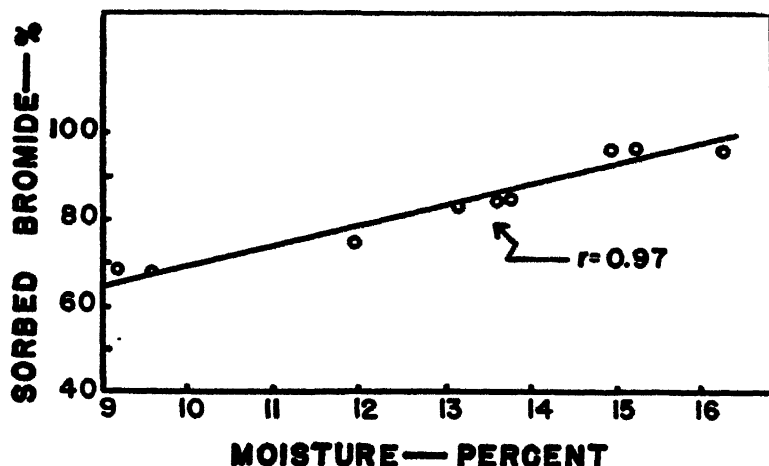


Fig. 2. Correlation between flour moisture content and sorbed bromide retained after aeration.

The hydrogen-ion activity of the fumigated flours was determined. Typical values are as follows:

Sample	pH value
Control.....	5.8
1b (dry; high bromide residue).....	5.4
4b (normal; lowest bromide residue).....	5.6
8b (wet; medium bromide residue).....	5.4

From these typical results it appears probable that the hydrogen-ion activity bears some relation to the bromide residue in the samples. However, the whole picture appears to be a balance between the chemical action of methyl bromide on the proteins; the effect of moisture on the hydrolysis of the fumigant; the effect of hydrolytic products on the flour properties; and the influence of the higher acidity due to the presence of these products.



TABLE I  
FLOUR ABSORPTIONS AND VALORIMETER READINGS DETERMINED WITH THE  
FARINOGRAPH ON FUMIGATED AND AERATED SAMPLES

Sample no.	Absorption <sup>1</sup> (%)	Valorimeter reading
Control	61.1	63
1 fumigated	61.3	60
1 aerated	61.0	62
2 fumigated	59.8	55
2 aerated	59.9	59
3 fumigated	60.5	60
3 aerated	61.0	62
4 fumigated	61.3	62
4 aerated	61.5	62
5 fumigated	60.4	61
5 aerated	60.4	62
6 fumigated	61.3	60
6 aerated	61.4	58
7 fumigated	61.9	59
7 aerated	61.9	60
8 fumigated	62.2	58
8 aerated	61.2	53
CO <sub>2</sub> -CH <sub>3</sub> Br fumigated	60.8	62
CO <sub>2</sub> -CH <sub>3</sub> Br aerated	60.5	65
Flour + cystine	61.1	56
Flour + tryptophane	61.1	58
Flour + tyrosine	61.1	56

<sup>1</sup> Absorption required to obtain a dough consistency of 500 Brabender units.

The samples were baked and the finished loaves were scored for odor, grain, and texture. Loaf volume data are plotted in Figure 3 and the regression line and regression coefficient were calculated ( $r = -0.85$ ). These results are sufficiently significant to indicate that increasing bromide content results in decreased loaf volume. Samples with relatively low bromide content had a less intense odor than the other samples, and the samples with the least bromide residue showed the best grain and texture.

*Fumigation with Carbon Dioxide-Methyl Bromide.* It was thought that it might be possible to use some other gas to react selectively with the proteins of flour and thus prevent methyl bromide from reacting. Carbon dioxide was tried in an experiment because its use in mixtures with other fumigants had been recommended.

Results for bromide residues are shown in Figure 1 as two isolated points. It should be noted that the values obtained are higher than the corresponding values, at similar moisture content, when bromide is used alone. But Figure 1 shows that the percentage of bromide sorbed in fumigation that was retained after aeration agreed with the values obtained with methyl bromide alone.

The addition of carbon dioxide to the fumigant does not appear to reduce the sorption of methyl bromide; probably this inertness

nullifies its use to prevent alterations of a chemical nature with methyl bromide. The farinograms obtained with these samples are shown in Figure 4.

*Addition of Amino Acids.* It is probable that reactions of methyl bromide with the flour proteins are dependent on the presence of a specific group in the molecules of amino acids, or on a specific amino acid itself. In order to throw some light on the latter assumption, 0.1% cystine, 0.1% tryptophane, and 0.05% of a crude preparation

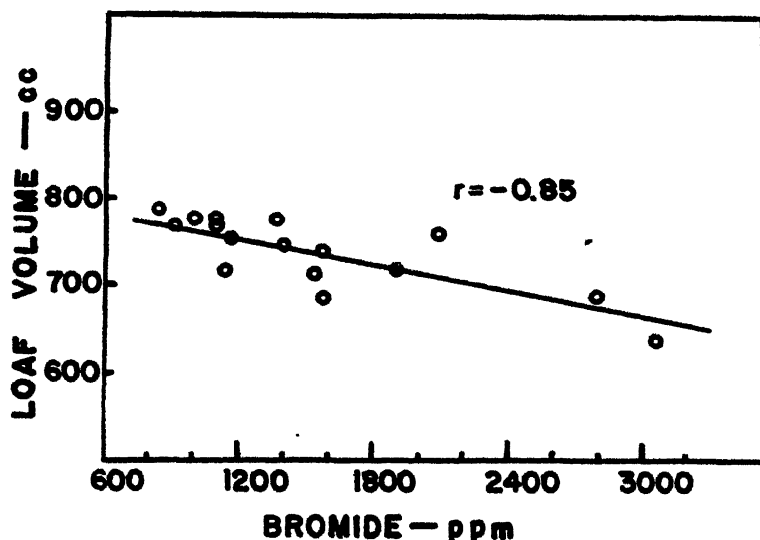


Fig. 3. Correlation between loaf volume and bromide sorbed.

of tyrosine were added to three samples of flour (13.3% moisture). They were then fumigated in the usual manner. The farinograms shown in Figure 4 do not indicate greater alterations than were obtained with fumigated flour.

Aqueous solutions of methionine and tryptophane were treated with liquid methyl bromide, the solutions added to nonfumigated flours, and the flours baked. Methionine-treated flour gave loaves with a very strong objectionable odor, while the tryptophane-treated flour yielded normal bread. But the same odor obtained with treated methionine was produced when a plain water solution of the amino acid was used. It is evident that the objectionable odor in this experiment was due to decomposition of methionine, and not to methyl bromide action.

*Treatment of Fumigated Flours with Oxidizing Agents.* Farinograms and mixograms prepared with fumigated flours resembled those

obtained with the addition of reducing substances like cysteine. For this reason the action of oxidizing agents on the pattern of the curves was determined. Sample 8 (Table I) was used for these experiments.

Farinograph curves were made with the appropriate amount of sample, and with added iodine, potassium bromate, and potassium

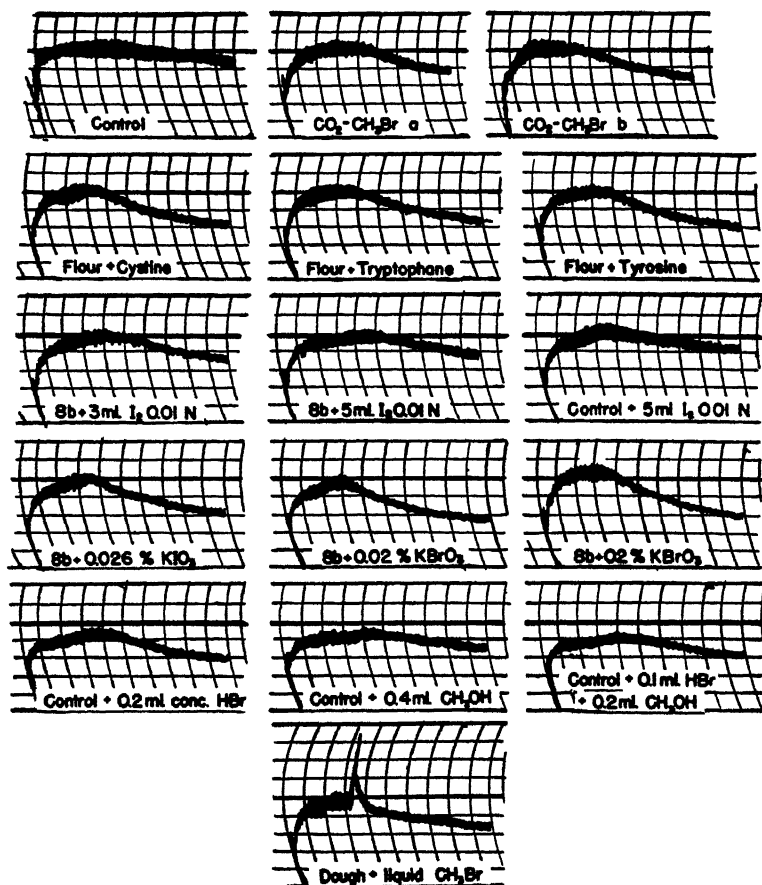


Fig. 4. Changes in the farinogram patterns produced by various substances. Each vertical line equals two minutes. The heavy horizontal line equals 500 Brabender units.  $\text{CO}_2\text{-CH}_3\text{Br}$  a and  $\text{CO}_2\text{-CH}_3\text{Br}$  b represent the same flour sample which contained 13.1% moisture and retained 0.17% and 0.14% Br before and after aeration. The curves shown in the bottom row were obtained when the reagents indicated were added to the water used in the farinograph.

iodate. The curves obtained for these variously treated fumigated flours are presented in Figure 4. Addition of 3 ml. of iodine in potassium iodide (0.01 N) resulted in a curve with good recovery. A better recovery was obtained after using 5 ml. of the same solution. On the other hand, 0.026% of potassium iodate and either 0.02% or 0.2% of potassium bromate did not show satisfactory improvement.

The favorable effect obtained with iodine solution might be due to the slight stiffening effect on the dough. In fact, nonfumigated flour treated with 5 ml. of the iodine solution showed that the dough had been toughened (Figure 4).

*Treatment of Flour with Hydrobromic Acid and Methyl Alcohol.* Since hydrobromic acid and methyl alcohol are possible hydrolytic products of methyl bromide, it seemed desirable to observe the effect of these compounds on flour. Hydrobromic acid produced a graph that resembles those obtained with fumigated flours. Methyl alcohol decreased the consistency of the dough but did not produce as fast a break as did methyl bromide. Incorporation of both agents together yielded an additive effect.

A nonfumigated flour treated with 0.5 ml. of concentrated hydrobromic acid and one treated with 0.5 ml. of methyl alcohol were baked according to the procedure already outlined. Neither one of these reagents produced loaves with objectionable odors, although they showed a detrimental effect on the volume. This was especially apparent with hydrobromic acid because it produced a decrease in loaf volume of 25%.

These results might suggest that methyl bromide has a twofold effect on flour. One is the specific reaction of the organic halide with a certain part or parts of the protein-containing fraction. The other is the action of the hydrolysis products, mainly hydrobromic acid, on the physical dough properties.

*Treatment of Dough with Liquid Methyl Bromide.* Reactions between methyl bromide and gluten proteins might occur at those parts of the molecules which are responsible for the formation of gluten. A study was therefore made to determine whether or not the fumigant could react with the proteins *after* the gluten network was established. Figure 4, bottom curve, shows the curve produced when the dough was treated with liquid methyl bromide just as it reached its peak consistency in the mixer. The sudden increase in consistency may be due to the cooling action of evaporation.

*Gas Production and Gas Retention.* It was of interest to determine whether or not bromine residues in methyl bromide fumigated flours affect the yeast used in baking and also if the ability of the gluten network to retain the carbon dioxide produced during fermentation is influenced. Measurements of the rate of gas production from a dough furnish information on the fermentation activity of the dough. Gas retention is useful to determine, roughly, alterations in the physical properties of the proteins. In the former determination the total volume of carbon dioxide produced during fermentation is measured, while in the latter only the gas retained in the dough is evaluated.

A hard winter wheat straight grade bakers' type flour with a moisture content of 13.9% was fumigated with methyl bromide at a dosage of 25 pounds per 1,000 cubic feet for 24 hours. The bromide sorbed after aeration was 0.086%. The fumigated sample gave a longer period of constant fermentation rate than the nonfumigated. The percentage of total gas produced which is retained by the dough showed a small decrease for fumigated flour, and in this respect confirms what was found by the baking tests.

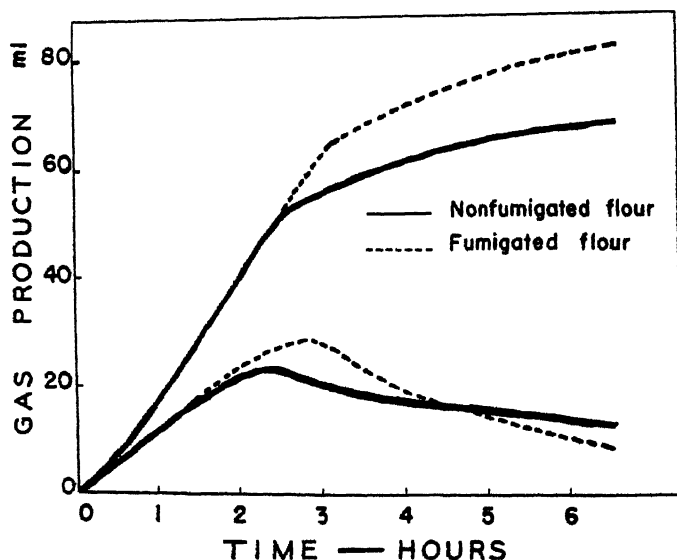


Fig. 5. The effect of excess methyl bromide fumigation on the gas production and gas retention properties of flour. Upper curves show gas production and lower curves show gas retention.

**Selenium-Containing Flours.** It is well known that selenium compounds tend to form products with very sharp odors. To determine whether a selenium-bearing flour showed more pronounced odors than a normal flour, a wheat containing 26.6 p.p.m. of selenium was milled, and two samples of the flour were fumigated with methyl bromide, one with 2 pounds per 1,000 cubic feet and the other with 25 pounds per 1,000 cubic feet. Both samples were baked. The odor of the loaves was no more objectionable than that of common flours fumigated at the same dosages.

**Germicidal Effect of Methyl Bromide.** During the course of this work it was found that flours fumigated with methyl bromide appeared to be relatively free from microflora. In order to corroborate this observation microfloral counts were made.

The results obtained, although of a preliminary nature, are considered important and significant. In these experiments four samples

of the same flour were used for assay of bacteria and fungi. Two of the samples had been fumigated with methyl bromide at dosages of 2 and 25 pounds per 1,000 cubic feet, another one had been treated with a similarly heavy dosage of methyl chloride, and the fourth was a nonfumigated control. The data obtained are presented in Table II and show that even a moderate dosage of fumigant had a pronounced microbicidal effect. Methyl chloride likewise appreciably decreased the number of microorganisms per gram.

TABLE II  
MICROFLORAL COUNTS ON FUMIGATED FLOURS

Treatment of sample	Incubation: 4 days; dilution 1/100 microorganisms per gram	
	Nutrient agar	Rose Bengal <sup>1</sup>
Check	approx. 30,000 <sup>2</sup>	1,700-2,700
Methyl bromide		
25 lbs./1,000 cu. ft.	0-100 <sup>2</sup>	0-300
2 lbs./1,000 cu. ft.	200-400	0-100
Methyl chloride		
25 lbs./1,000 cu. ft.	700-1,200	100-300

<sup>1</sup> Rose Bengal medium preferentially inhibits bacteria growth.

<sup>2</sup> Incubation period: 2 days.

### Discussion

All the evidence gathered during the various stages of this study indicates that methyl bromide reacts with the protein fractions of flours. Methyl chloride is also able to react with flour. However, the products of these two reactions apparently are different, because the odors of the loaves baked from the treated flours are unlike.

A reaction similar to that of methyl bromide on the proteins of flour occurs with other proteins. Of a number of proteins investigated, gelatin was the only one to give a negative reaction when exposed to methyl bromide and treated with alcoholic potassium hydroxide. Gelatin does not contain the amino acids valine, beta-hydroxyglutamic acid, tyrosine, methionine, and tryptophane. This deficiency may be significant as an indication that one or more of the amino acids missing in gelatin are responsible for the odor produced when methyl bromide reacts with protein.

The moisture content is important in the sorption of methyl bromide by flour, since it appears to control both the amount of fumigant fixed as well as its mode of action. When moisture is low, methyl bromide is bound mostly by chemical reactions with the protein fractions; conversely, when moisture is high it hinders these reactions and favors hydrolytic phenomena. The products of these two modes of action are different and the effects also are different. It is of par-

ticular significance that minimum sorption of the bromide takes place in the neighborhood of 14% moisture (Figure 1).

It is evident that the high bromide residues resulting from overfumigation decrease the baking quality of flour. This is true not only because of the objectionable odor that develops in the baked loaves but also because of alterations in the physical properties of the dough. Accordingly, methyl bromide fumigation of flour should be performed with considerable care to avoid overfumigation.

One of the objectives of this work was to find some means to avoid the bad effects of overfumigation. However, no way has been discovered to alleviate the damage to the flour once overfumigation occurs. Aeration, of course, helps to eliminate the volatile fumigant that has not reacted with the flour, but aeration does not change the amount of sorbed methyl bromide.

Further work should be directed toward determining the reaction between methyl bromide and cereal proteins. If the reaction were known it might be possible to correct the difficulty, although this research appears to indicate that the reaction is severe enough to alter the protein irreparably. It was thought that the fumigant might attack the disulfide linkages ( $-S-S-$ ) and liberate sulfhydryl groups ( $-SH$ ). This should weaken the protein structure, especially the cross linkages, and at the same time the sulfhydryl groups should contribute to the off-odors which appear in baking. The nitroprusside test, however, has shown that there is no liberation of sulfhydryl groups, even if the fumigant acts directly on an amino acid like cystine or methionine. The latter amino acid was chosen because, as previously noted, it is not present in gelatin. Further, the  $-SCH_3$  group of this amino acid could conceivably react with methyl bromide to give an addition compound (sulfonium compound) which could be decomposed by heat. However, these experiments did not confirm this possibility. Furthermore, it was found that when cysteine, which contains a free  $-SH$  group, is used in baking it markedly affects the baking quality of flour but does not give an objectionable odor.

Tryptophane was another amino acid tested because of its absence in gelatin and because of its intimate relation with skatole and other indole derivatives which are usually very odoriferous. It also failed to give any reaction with the fumigant.

During the fermentation of doughs made up from nonfumigated flour the normal microflora may use part of the sugar present for their own metabolism. With destruction of the microflora this sugar would remain in the doughs and be available to the yeast. The longer maintenance of peak fermentation rate with the fumigated flour could therefore be explained in this manner. However, a more logical ex-

planation is on the basis of the stimulation of yeast activity. On the other hand, a change in the starch-amylase relationship in the dough due to methyl bromide fumigation is not excluded. No investigations have been made in this direction, but it is possible that the bromide reaction could result in either the starch being made more available to hydrolysis or the amylases become more active. In either case there would be a net increase in the amount of fermentable sugar.

Complete or partial elimination of the microorganisms present in flour would be of great value in preventing the development of "rope" in bread. In future studies with methyl bromide fumigation of flours it would be very desirable to determine whether or not flours fumigated at normal dosages can develop rope. Thus an important contribution to the baking industry might be made. Furthermore, methyl bromide could possibly be used as a tool for disinfection of materials when heat cannot be employed.

The effects of methyl bromide fumigation on flour properties therefore are twofold. If used in excessive amounts, it is detrimental to baking quality. On the other hand, in addition to serving as an insecticidal agent, it appears to have definite promise as an effective germicide.

### Summary

The effect of excessive methyl bromide fumigation of flour on the physical properties of doughs, the quality of bread produced, and the differential effect of various flour constituents were investigated.

Fumigation with normal concentrations of methyl bromide (1 to 2 pounds per 1,000 cubic feet) causes no lasting deleterious effects on flour. However, at high concentrations such as used in this study (25 pounds per 1,000 cubic feet) irreversible damaging changes occur. Tests showed that the changes are associated with the gluten protein fractions of flour. Excessive methyl bromide treatment of flour reduces dough development time and lessens mixing tolerance, as indicated by dough development curves. The loaf volume of bread made from treated flour is reduced and the bread produced has an undesirable odor. The importance of avoiding the overfumigation of flour is apparent.

Moisture content was shown to be of primary importance in controlling the sorption of methyl bromide. At moisture values below 14% chemical action on proteins is involved, whereas at high moisture values the effects appear to be associated with hydrolytic products of the fumigant.

Methyl bromide appears to be an effective germicidal agent for flour.



### Acknowledgments

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# A SIMPLE SEDIMENTATION TEST FOR ESTIMATING THE BREAD-BAKING AND GLUTEN QUALITIES OF WHEAT FLOUR

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A test for estimating potential bread-baking quality, that is more simple, rapid, and practical than the Kjeldahl protein test or other tests now used to evaluate wheat in terms of baking quality, would be extremely valuable for use in the grading of wheat. To be practicable for this purpose a test should be simple enough to be applied by grain inspectors who have not had chemical training, should be rapid enough not to delay grading seriously, should require no expensive or elaborate equipment, and should predict wheat quality at least as accurately as existing tests.

The conventional Kjeldahl protein test, used to evaluate wheat, does not meet these requirements for a practical test, particularly in respect to the simplicity of equipment needed and technical training necessary. Furthermore, the Kjeldahl protein test does not adequately reflect the bread-baking potentialities of wheat that has inferior gluten quality as a result of unfavorable environmental conditions during growth, damage in storage, or because it is of a variety having inherently inferior gluten quality.

It has long been known that differences among flours from different types of wheat are reflected by the abilities of the gluten proteins to imbibe water. The relationship between the colloidal swelling of gluten and the bread-baking quality of flour was probably first reported by Upson and Calvin (1916). Gortner and Doherty (1918), in studying the rate and extent of the swelling of gluten disks in dilute solutions of various acids, found that glutens from "strong" flours have much higher rates of hydration and much higher hydration capacities than do glutens from "weak" flours. Lüers and Ostwald (1920) and Gortner and Sharp (1923) demonstrated the relationship between flour baking strength and hydration capacity as measured by the viscosity of acidulated suspensions of flour in water. Lüers and Schneider (1921), in comparing various methods for determining the hydration capacity of colloids, found good agreement among Hofmeister's method of weighing before and after the imbibition of water by the colloid, Fischer's method of determining the change in volume, and the viscosity method.

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Numerous investigations have been made in recent years on the viscosity of flour-water suspensions, and viscosity measurements have been found useful in evaluating soft wheat flours. Finney and Yamazaki (1946), in applying viscosity measurements to hard wheat flours, found that for individual varieties there is an essentially linear relationship between viscosity and protein content, but that this relationship is not the same for different varieties and that these varietal differences cannot be properly evaluated by means of viscosity measurements.

Finney and Yamazaki (1946), in further investigations, however, developed a method of testing by which the water-retention capacity of hard wheat flour is measured by determining the weight of material separated from an acidulated flour-water suspension by means of centrifugal force under prescribed conditions. The values obtained correlated well with bread loaf volumes and appeared to evaluate the various varieties of hard red winter wheat properly in terms of their gluten quality.

The United States Department of Agriculture is carrying on several lines of research in an effort to devise a suitable practical test that may be used in connection with the official inspection of wheat and that will reflect with at least reasonable reliability the baking quality of the flour that can be milled from the wheat. This is a preliminary report of a somewhat novel approach to the problem which has progressed far enough to be of general interest. The test described is based on the rate of sedimentation of the solid phase from an acidulated suspension of flour in water. The test has so far been applied only to white flour, but it is anticipated that further research will result in its application either to whole wheat meal or to a crude white flour quickly prepared without elaborate milling.

### Method

In an effort to adapt measurements of water imbibition to a procedure that would meet the previously mentioned requirements of a practical grain inspection test, it was noted that the rate at which the solid phase of an acidulated water suspension of whole wheat meal settles to the bottom of a container varies greatly among different samples of wheat. Rapid settling of such suspensions was found to be associated with low protein content and with wheat varieties having poor gluten quality. Conversely, slow settling was observed to be associated with high protein content and good gluten quality. The solid phase of this type of suspension appears to consist of a mass of greatly swollen gluten particles in which are imbedded most of the other insoluble constituents of the wheat. The level to which the solid phase will settle under the

force of gravity in a given interval of time depends largely on the quantity of swollen gluten present and on the degree of swelling. The greater the amount of water imbibed by the gluten the lower will be the specific gravity of the swollen gluten and the slower will be the rate at which it will settle. Attempts to apply this principle to the testing of whole wheat meal immediately brought to light the fact that variations in the method of grinding the wheat may greatly affect the results obtained. In order to eliminate as far as possible this variable factor until the possible usefulness of the principle itself could be further investigated, the present studies have been confined exclusively to experimentally milled, unbleached, unenriched white flour.

The following procedure was applied to all the flour samples tested:

Place a quantity of flour equivalent to 4.00 g on a 14% moisture basis in a 100 ml glass-stoppered graduated cylinder having a distance of from 180 to 185 mm between the zero and 100 ml marks. Add 50 ml of distilled water to the cylinder, shake the mixture for 30 seconds, and allow it to stand for 5 minutes. Add 25 ml of dilute lactic acid,\* then mix the contents of the cylinder by inverting the stoppered cylinder and returning it to the upright position 10 times. (Do not shake the cylinder.) Immediately after mixing place the cylinder in an upright position and start timing with a stopwatch or interval timer. After an interval of exactly 5 minutes read the volume of the solid phase of the material in the graduate. This volume in milliliters is the "sedimentation value" of the flour.

Figure 1 shows the appearance of the sedimentation tubes at the time readings are taken. The line of demarcation between the solid and liquid phases is ordinarily sharp and distinct, as shown in the figure, so that readings may be made to the nearest milliliter and estimated to the nearest 0.1 ml. Occasionally the line of demarcation is less distinct but rarely is it so indistinct that readings may not be made to the nearest milliliter. Duplicate determinations usually agree within less than 1 ml. In a series of 135 samples of flour tested in duplicate, the average difference between duplicates was 0.5 ml and the maximum difference was 2.5 ml. Temperature of reagents within the range of 20°C to 30°C has little effect on the results. Minor variations in strength of acid or in the time and manner of shaking or mixing have no appreciable effect on the results. The 5-minute period of settling, however, must be accurately timed, since at that time sedimentation is still progressing fairly rapidly. Readings taken at longer time intervals show smaller differences between good and poor bread flours, and readings taken at shorter time intervals tend to be somewhat erratic. Sedimentation values can be expected to range from 20 or less for low protein flour of very inferior bread-baking

\* The dilute lactic acid is prepared by diluting 250 ml of 85% lactic acid to 1000 ml. The diluted acid must be allowed to stand for at least 3 weeks before use, or refluxed at its boiling temperature for 6 hours without loss of volume and cooled to room temperature before use. The reagent thus prepared will keep indefinitely without change in strength.

quality to 55 or more for high protein flour of superior bread-baking quality. For comparative purposes the test should be applied to the same grade of flour (straight, long patent, short patent, etc.) since differences in flour ash content have some effect on the sedimentation values.

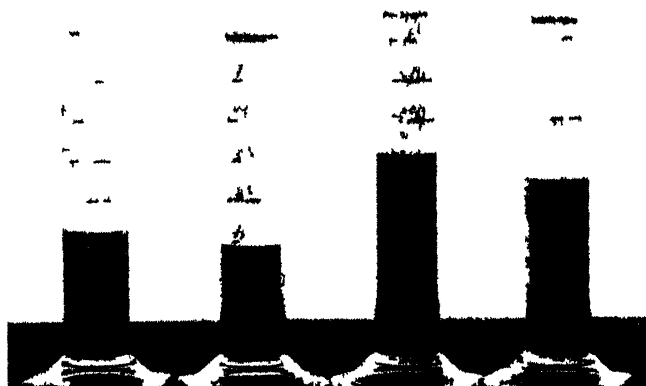


Fig 1 Appearance of sedimentation tubes at the time of reading

### Materials

The sedimentation test was applied to 135 samples of hard wheat flour that had previously been milled and baked in connection with other research projects. These flour samples were milled experimentally from 52 samples of hard red winter wheat of known pure varieties grown in experimental plots at five stations in four states, six samples of commercially grown hard red winter wheat of known variety, 59 samples of commercial hard red winter wheat consisting mostly of mixtures of varieties that were tentatively identified from the physical characteristics of the kernels, and 18 samples of hard red spring wheat of known varieties grown in experimental plots at two stations.

The wheats were milled to 90% patent flours on the Allis-Chalmers experimental flour mill. The average ash content of the flours was about 0.42% (14% moisture basis). The bread-baking tests were made by a formula described by Fifield *et al.* (1945) using 100 g of flour, 2.0 g of compressed yeast, 1.5 g of salt, 5.0 g of sugar, 0.25 g of malted wheat flour, 3.0 g of shortening, 4.0 g of nonfat dry milk solids, and varying amounts (0 to 4 mg) of potassium bromate. The ingredients for two loaves were mixed together for a sufficient length of time for proper dough development by using a Hobart-Swanson dough

mixer with four pins in the head and two pins in the bowl and operated at 108 r.p.m. The doughs after mixing were divided into two equal parts, fermented for 3 hours at 30°C, panned and proofed for 55 minutes at 30°C, then baked for 25 minutes at 232°C. For the purposes of this study the loaf volume data in each instance is that of the loaf containing the amount of potassium bromate that produced the greatest loaf volume. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color.

### Relation Between Sedimentation Time, Loaf Volume, and Protein Content

Average data for loaf volume, protein content, and sedimentation value of the 135 flour samples classified according to variety or type are given in Table I. The relationships between protein content and

TABLE I  
AVERAGE LOAF VOLUME, PROTEIN CONTENT, AND SEDIMENTATION  
DATA FOR 135 SAMPLES OF HARD WHEAT FLOUR

Variety or type	No. of samples <sup>1</sup>	Average loaf volume	Average protein content <sup>2</sup>	Average sedimentation value	Average specific loaf volume	Average specific sedimentation
		<i>ml</i>	<i>%</i>	<i>ml</i>	<i>ml</i>	<i>ml</i>
Turkey (pure variety)	7	720	11.7	39.2	48.4	3.3
Wichita (pure variety)	6	748	12.3	41.4	48.5	3.3
Tenmarq (pure variety)	7	738	11.9	38.7	48.6	3.2
Pawnee (pure variety)	7	752	12.5	38.9	47.9	3.1
Comanche (pure variety)	7	743	12.4	44.4	46.9	3.6
Early Blackhull (pure variety)	5	670	11.6	35.3	45.0	3.0
Blackhull (pure variety)	6	706	12.3	35.9	44.9	2.9
Red Chief (pure variety)	7	599	11.6	29.2	37.7	2.5
Chiefkan (pure variety)	6	625	12.4	29.2	37.5	2.4
Commercial HRW (predominantly "desirable" varieties)	49	641	10.4	33.2	46.3	3.2
Commercial HRW (predominantly Red Chief or Chiefkan)	10	538	9.7	26.4	39.1	2.7
Hard red spring wheat <sup>3</sup>	18	819	12.6	43.9	52.2	3.5

<sup>1</sup> In the instance of each of the named pure varieties each sample was grown at a different station.

<sup>2</sup> Calculated to a 14% moisture basis.

<sup>3</sup> Pure varieties grown at two different stations.

loaf volume and between sedimentation value and loaf volume are shown graphically by means of scatter diagrams and regression lines in Figures 2 and 3 respectively.

The values obtained from statistical analysis of the data are given in Table II. From the correlation coefficients and standard errors of estimate it is evident that for this particular series of flour samples sedimentation rate is fully as good as protein content as an index of

bread loaf volume. From the scatter diagram in Figure 2 it may readily be seen that if the regression equation were to be used to predict loaf volume from protein content, the loaf volumes predicted for flour milled from Chiefkan or Red Chief wheat (varieties of generally recognized inferior gluten quality) would be expected to be consistently greater than the loaf volumes actually attained and the errors of prediction would in some instances be very large. Referring then to Figure 3, it is obvious that by using the sedimentation value the loaf volume of flour from Chiefkan and Red Chief wheat can be predicted on the same basis and with essentially the same degree of reliability

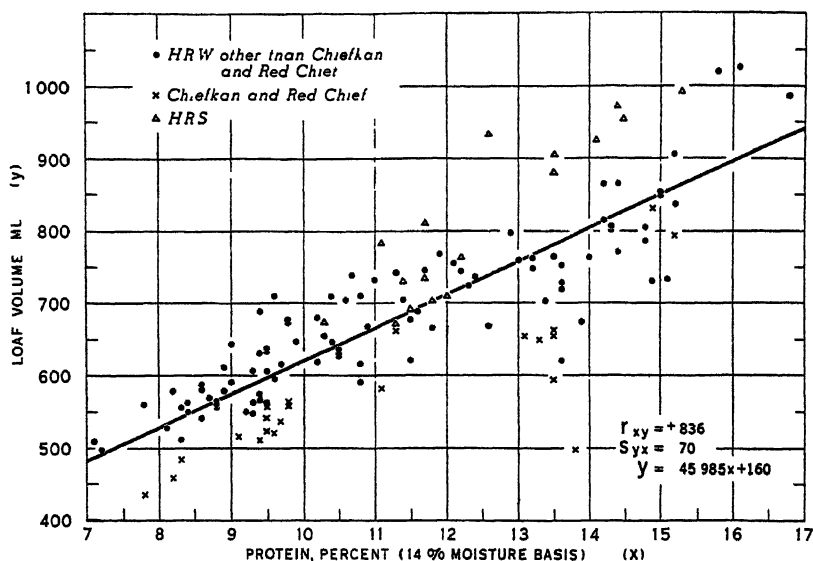


Fig. 2. Relation between protein content (14% moisture basis) and bread loaf volume.

as that of bread from other varieties of hard wheat. The sedimentation value tends to evaluate flour from Chiefkan and Red Chief wheat properly in comparison with flour from other varieties of hard wheat, while the protein test, on the other hand, almost invariably fails to reflect the relatively poor bread-baking quality of flour from these two varieties of wheat.

In the series of 135 samples of flour under investigation 17% of the samples consisted of flour milled from wheat entirely or predominantly of varieties of recognized inferior gluten quality. It is reasonable to assume that the greater the percentage (up to 50%) of such inferior gluten quality samples in a series, the lower would be the correlation between protein content and loaf volume for the entire series and

the greater would be the advantage of sedimentation value over protein content as a measure of loaf volume.

TABLE II  
STATISTICAL ANALYSIS OF DATA

Correlation coefficients <sup>1</sup>	Standard errors of estimate	Coefficients of partial correlation
$r_{pv} = .836$	$S_{sp} = 70$ ml.	$r_{pr s} = .497$
$r_{sv} = .863$	$S_{is} = 64$ ml.	$r_{sv p} = .604$
$r_{sp} = .790$	$S_{ps} = 1.42\%$	$r_{sp v} = .249$

<sup>1</sup>  $p$  = percent protein,  $s$  = sedimentation value,  $v$  = bread loaf volume in milliliters.

The coefficient of partial correlation  $r_{sv.p}$ , having a value of .604 and showing the relationship between sedimentation value and loaf

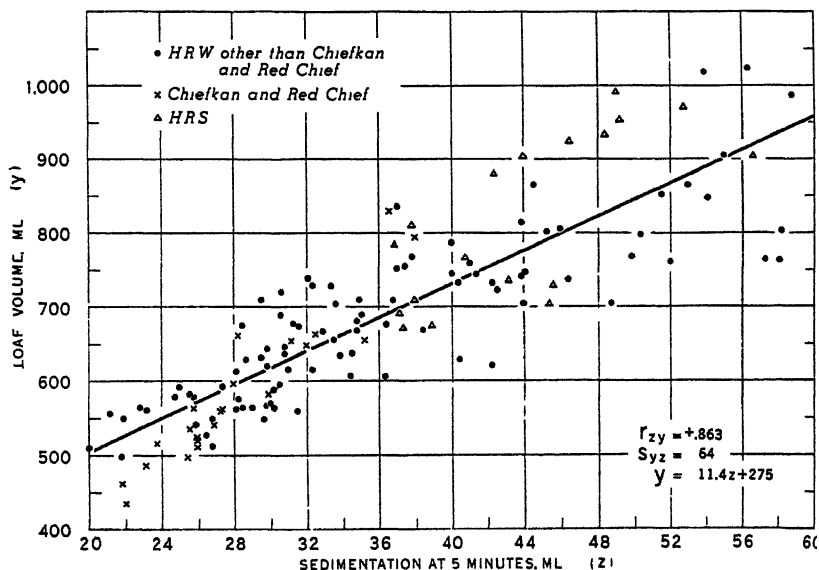


Fig. 3. Relation between sedimentation value and bread loaf volume.

volume independent of protein content, indicates that at a constant protein content level sedimentation value is significantly correlated with loaf volume. Then since differences in loaf volume at any protein content level are assumed to be caused by differences in gluten quality, the observation that sedimentation value is partially and significantly dependent upon gluten quality is supported by statistical evidence.

### Specific Sedimentation as a Measure of Gluten Quality

Since the sedimentation value depends both on the quantity and quality of the gluten in the flour it should be a better index of potential



bread loaf volume than a test which measures only gluten quantity or gluten quality. In order to express the results of the sedimentation test in terms that tend to reflect only gluten quality, however, the sedimentation value in each instance was divided by the percentage of protein (on a 14% moisture basis) in the flour and the resulting value, for convenience, is called the "specific sedimentation."

Loaf volume, likewise, depends both on the quantity and quality of the gluten in the flour. In order to express loaf volume data in terms that tend to reflect only gluten quality, a value known for the purposes of this study as "specific loaf volume" was calculated by the formula:

$$V_s = \frac{V - K}{P}$$

where  $V_s$  = specific loaf volume

$V$  = actual loaf volume in ml

$K$  = theoretical volume of a loaf made from flour containing no protein

$P$  = percent of protein in the flour (14% moisture basis).

The value of  $K$  was determined by extrapolating the regression line of loaf volume against protein percentage to a protein value of zero percent. The value obtained was 160 ml and closely approximated the actual loaf volume obtained by baking a loaf of bread in which starch was substituted for the flour in the bread formula.

Data for specific loaf volume and specific sedimentation are given in the last two columns of Table I.

A graphic comparison of the specific loaf volume and the sedimentation value methods of expressing gluten quality is shown in Figure 3. For the purpose of this comparison the averages of the specific loaf volumes and of the specific sedimentation values for each variety or other group of flour samples are compared with the corresponding average values for the 45 samples of flour from experimentally grown hard red winter wheats of known "desirable" varieties, which values are both arbitrarily taken to be 100. It is interesting to note that the individual varieties of hard red winter wheat, with the exception of the variety Comanche, are rated in essentially the same order by both methods of estimating gluten quality. The generally recognized marked inferiority of the gluten of Chiefkan and Red Chief wheat is fully reflected both by the specific loaf volume and specific sedimentation values. The sedimentation test appears to indicate that the gluten from Comanche flour is rather distinctly superior to that from any of the other "desirable" varieties of hard red winter wheat, a finding that is not confirmed by the baking tests. This suggests the possibility that flour

from Comanche wheat may have a bread-baking potentiality not fully reflected by our usual baking tests.<sup>3</sup>

The 23 samples of flour from wheat consisting entirely or primarily of the varieties Chiefkan or Red Chief were found without exception to have specific sedimentation values of less than 3.0. Of the 112 samples of flour from wheat consisting entirely or primarily of varieties other than Chiefkan or Red Chief, 91 were found to have specific sedimen-

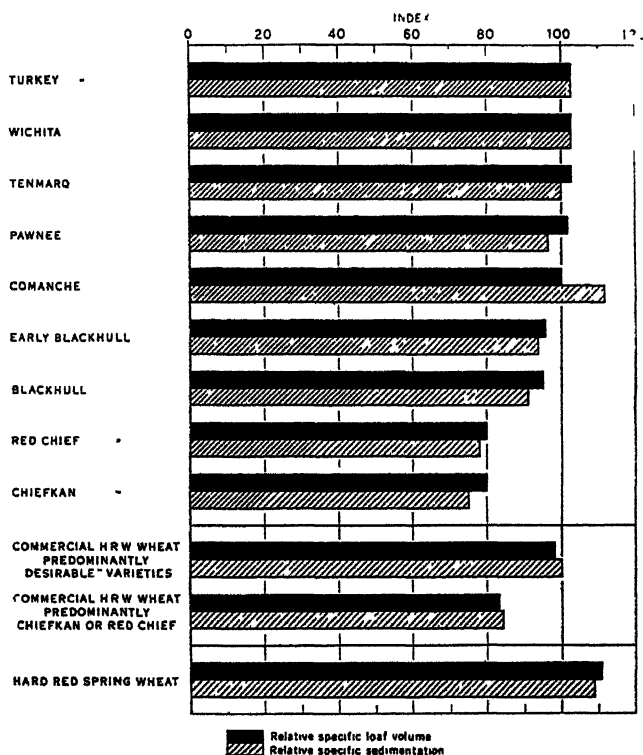


Fig. 4. Relative gluten quality of different varieties of hard red winter wheat, commercial lots of hard red winter wheat, and variety samples of hard red spring wheat as measured by specific loaf volume and specific sedimentation.

tation values of 3.0 or higher, and of the 21 samples of this group that had specific sedimentation values of less than 3.0, all but seven were shown by the specific loaf volume data to have gluten of less than average quality. From the data obtained, therefore, it seems proper to conclude that for experimentally milled hard wheat flour from the 1946 crop, it should be possible to segregate with a reasonable degree

<sup>3</sup>The data in respect to the comparative bread-baking quality and gluten quality of different varieties and classes of wheat presented in this paper are intended only for the purpose of comparing different methods of testing. The data are grossly inadequate in quantity to be used in any attempt to establish the relative quality of different varieties or classes of wheat and should, therefore, not be considered from that standpoint.

of accuracy flour from the varieties Chiefkan and Red Chief along with any other flour of markedly inferior gluten quality by means of the specific sedimentation value.

### Summary

A quick sedimentation test of extreme simplicity, in which the rate of sedimentation of the solid phase of an acidulated suspension of flour in water is measured, has been devised for estimating the bread-baking quality of flour.

In a series of 135 experimentally milled flours representing individual varieties of hard red winter and hard red spring wheat grown at different stations and commercial hard red winter wheat from different markets, the sedimentation value was found to be fully as good an index of the bread loaf volume as was the protein content.

Flour from Chiefkan and Red Chief wheat (varieties of generally recognized inferior gluten quality) tends to be properly evaluated in respect to potential bread loaf volume by the sedimentation test, while the protein test almost invariably overestimates (often greatly) the bread loaf volume that can be attained from such flour.

Specific sedimentation (sedimentation value divided by protein percentage) is a useful measure of gluten quality. In the series of 135 flour samples, the inferior gluten quality of the 23 samples of Chiefkan or Red Chief flour (or flour milled from wheat consisting predominantly of those varieties) was reflected in every instance by the specific sedimentation. The relative gluten qualities of flour from nine of the leading commercial varieties of hard red winter wheat were, with one exception, evaluated in essentially the same order by their specific sedimentation values as by their specific loaf volume values.

### Acknowledgments

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## PROPORTION OF HULL IN SOME NORTH DAKOTA BARLEY VARIETIES, AS DETERMINED BY THE AIR JET TECHNIQUE

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The hull component of the barley kernel is of little use for feeding purposes. It is, nevertheless, a substantial portion of the threshed grain, constituting as much as 10% or more of the whole kernel. A knowledge of the hull percentages in various barley varieties is important to the breeder who wishes to develop varieties relatively low in hull content. Information regarding hull percentages has been difficult to secure because of the labor and time required to determine hull values by manual methods.

Fraser (1944) described an air jet technique for dehulling seeds which appeared to supply the answer to the problem of obtaining reliable information regarding hull percentage by a fairly rapid, simple method. Essentially the method consisted of violently agitating the soaked or tempered grain by an air jet while enclosed in a metal cup. The cup was  $2\frac{1}{4}$  inches in both length and diameter and was fitted for barley dehulling with a wire screen which lined the bottom, sides, and top of the cup. The top screen was removable. The jet was operated under a pressure of approximately 20 pounds per square inch. This caused the kernels vigorously to bombard the screen-covered confining walls of the cup, while the hulls were forced through the top screen, leaving the hulled kernels in the cup. Ten to 15 minutes sufficed to attain satisfactory removal of the hulls. The dehulled kernels were air-dried to remove moisture absorbed during the tempering period,

then weighed to ascertain the loss in weight due to dehulling. A 3-g charge was found to be quite satisfactory. This method gave very slightly lower hull percentages than the hand method, due to less complete removal of the hull from the crease of the kernel. Hannchen was especially difficult to dehull.

In view of the interesting data obtained by use of the air jet method in dehulling Western Canada barley, it was decided to construct a similar apparatus and employ it in ascertaining the hull content and ease of hull removal in several varieties of barley grown on experimental plots at various locations in North Dakota.

### Materials, Equipment, and Methods

*Materials.* The material used for the principal part of the investigation consisted of five varieties of barley grown at six stations in the state. The varieties and stations are identified in Table I. These barleys were grown under comparable conditions, using farm practices common in the area. Each sample was passed several times through

TABLE I  
AVERAGE VALUES OF HULL REMOVED BY ALL TESTS

Length of run	Varietal averages				
	Tregal	Kindred	Manchuria	Trebi	Plush
<i>Minutes</i>	%	%	%	%	%
5	8.5	7.7	7.4	6.5	6.6
10	11.0	10.7	9.8	8.4	8.5
15	11.7	11.2	11.6	10.1	10.1
20	12.8	12.6	11.8	10.9	11.0
Mean	11.0	10.5	10.1	9.0	9.0
Significant difference		5% level 1% level	1.10 1.46		

Length of run	Station averages					
	Langdon	Williston	Edgeley	Minot	Dickinson	Fargo
<i>Minutes</i>	%	%	%	%	%	%
5	7.1	8.1	7.1	7.7	7.7	6.3
10	10.5	10.3	10.0	9.8	8.9	8.6
15	11.9	11.3	11.4	11.1	10.2	9.7
20	12.5	11.2	12.2	11.4	11.6	12.1
Mean	10.5	10.2	10.2	10.0	9.6	9.2
Significant difference		5% level 1% level	1.06 1.34			

an Emerson dockage tester to remove extraneous material. In certain of the samples, barley kernels persisted in tailing over because of extremely long awns. These kernels were finally passed through a scouring machine and the long awns removed in this manner and then the kernels once more put through the dockage separator. The cleaned samples were used for test weight, moisture, protein, and hull determinations.

*Equipment* The apparatus used for dehulling was essentially that used by Fraser and had approximately the same dimensions (Figure 1)



Fig. 1 Apparatus used for dehulling barley kernels. The disassembled unit is shown at left with container, wire cage and cover while the huller ready for use is at the right

A metal cylinder closed at one end was fitted with a wire cage of slightly smaller size. The cage was constructed from No. 15 tempered steel wire flax screen. A cap was also made from this wire to close the container and prevent loss of kernels while permitting free egress to hulls. The container was held quite firmly in a hollowed-out wooden block hinged at the side and fastened during operation by a spring which hooked over a suitable pin. This permitted easy and rapid fastening and removal of the container. The inner portion of the block was lined with felt to assist in holding the container firmly during the dehulling operation.

As shown in Figure 1, two containers were used simultaneously with two brass air jets, 1.2 mm inside diameter. These branched from a central tube in the form of a "Y." The position of the air jets was quite critical because it affected the uniformity of dehulling. A compressor driven by an electric motor supplied compressed air at a line pressure of 17.5 pounds per square inch. Duration of dehulling

was controlled by an automatic timing device which is shown at lower right. It was found necessary to place the air jet nozzle carefully just inside the cover screen at the lower edge with the container lying on the side, as in the complete assembly pictured on the right of the figure. When the compressor motor was started, the kernels were violently agitated and thrown against the lining.

*Method.* Preliminary trials with barley tempered for various periods and temperatures failed to give entirely satisfactory results. It was difficult to secure satisfactory removal of the hull, and the hull values were low. This may have been due in part to absorption of water by the kernel during tempering, although a few samples of dehulled barley left exposed in the laboratory overnight did not change appreciably in weight. A few experiments with Aerosol (1% solution) failed to improve the results obtained by preliminary tempering. It was at first thought that the unsatisfactory results might be due to incomplete penetration of the water, and that this reagent would improve the ability of the water to soften the hull. Various tempering periods and temperatures were tried without appreciable success.

In view of these results it was decided to see what would happen if no initial tempering at all was used. Surprisingly good results were immediately obtained, which agreed closely with the values reported by Fraser, and there appeared to be little cause for further study of the effect of tempering. The temperature and humidity of the laboratory were fairly constant, averaging 80°F and 32% respectively. These factors, particularly relative humidity, should be taken into consideration when employing the air jet method.

Using the method without tempering, a moisture loss of 0.1 to 0.2% was occasioned by dehulling for 15 minutes, the larger loss occurring with barley containing 11.8% moisture. These changes are, of course, insignificant and accordingly the kernels could be weighed immediately after dehulling without the necessity of correcting for loss or gain in moisture content. Several determinations were made at higher relative humidities (58–59%) to ascertain their effect on hull removal. The samples were exposed to this humidity for one and one-half hours previous to dehulling. Hull removal was incomplete under these conditions. Apparently the relative humidity of the laboratory has a marked effect on the facility with which the hulls may be removed from barley.

The procedure finally adopted consisted of placing a 3-g charge of clean barley in the apparatus and starting the blast. Various lengths of time were used for dehulling to secure information on the relative rate of hull removal from various barley varieties. While one pair of samples was being dehulled, the next pair was weighed out. The

provision of two air jets and two barley dehullers of course doubled the number of samples handled in a specified time. After dehulling, the kernels were removed from the container, any loose hulls separated, and the kernels weighed to the nearest centigram. If the difference between duplicates exceeded 6 cg, the determination was repeated. No hand peeling determinations were made in view of the comparative data from the air jet and hand methods reported by Fraser.

### Results

Some of Fraser's data are reproduced below and compared with results secured by the present method:

	<i>Wt. of hulled barley, g</i>
University of Saskatchewan:	
Knife peeling	2.64
Air jet	2.67
North Dakota Agricultural Experiment Station:	
Air jet, 10 min.	2.74
Air jet, 15 min.	2.69
Air jet, 20 min.	2.67

The values are not strictly comparable because the varieties are not identical in the two sets of barley samples, and in addition they were grown under different environments. However, the results indicate very good agreement between the methods, particularly with the longer dehulling periods in the North Dakota modification. The hand method tended to give higher values because of more complete removal of hull from the crease, but the differences from the air jet method are very small and doubtless would not affect the comparative ranking of the varieties by the two procedures. The appearance of a representative sample of barley as dehulling proceeds is shown in Figure 2.

*Averages for Varieties and Stations.* The average percentage of hull removed from the varieties shows that there are substantial differences in this component among the five barley varieties (Table 1). The proportion of hull removed from each barley with increased length of treatment rose rather consistently for each variety with the greatest effect evident at the 20-minute period. The percent of hull removed with varying length of dehulling time is shown graphically in Figure 3. Tregal and Kindred show a distinct change in rate of hull removal after 10 minutes and Manchuria after 15 minutes, while the relation between hull removal and time is almost linear up to 15 minutes for Plush and Trebi. However, the lines bend at the 15-minute point, demonstrating a curvilinear effect. There is some indication of a slight change in the latter two curves after 15 minutes. The loss after



15 minutes may include some abrasion of the kernel itself although none was evident under slight magnification (Figure 2). The change in hull content with treatment is clearly shown in the photograph, with evidence of rather incomplete hull removal after 10 minutes. Characteristic differences in hull content are evident in Figure 3 and support the belief that barley varieties differ inherently in this component. Further conclusive evidence on this point is presented in the analysis of the data given in the following section. Tregal is quite similar to Kindred, and Trebi to Plush, in the proportion of hull, but there are very significant differences in hull content between the first and the

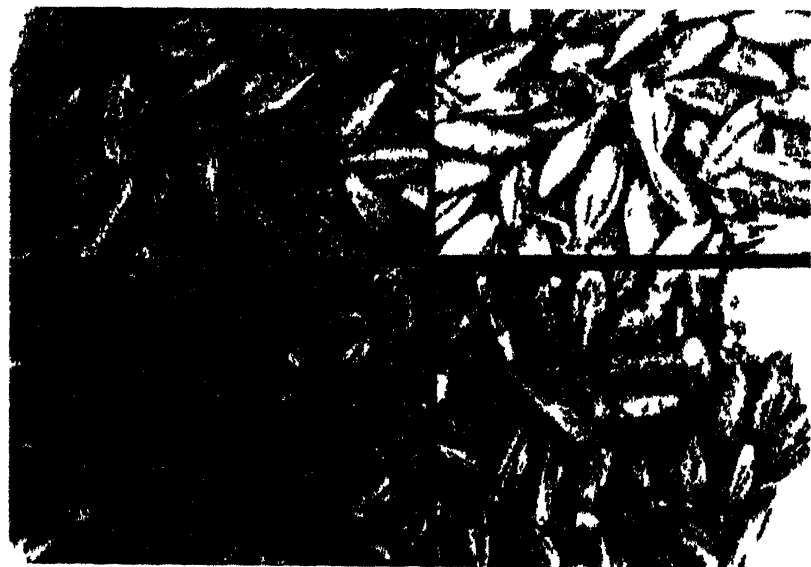


Fig. 2. Appearance of the barley kernels following different dehulling periods. Upper left, original sample before dehulling. Upper right, after 5 minutes' dehulling. Lower left, after 10 minutes' dehulling. Lower right, after 15 minutes' dehulling.

latter pairs. Manchuria is significantly higher than Trebi and Plush and lower than the others. The differences required for significance at the 5 and 1% levels are shown below the varietal averages. These were calculated from the analysis of variance shown in Table II.

Kernel size is very markedly affected by variety, and accordingly hull thickness may not vary directly with the percentage of hull. In the present study, for example, when the weight of hull per kernel for Manchuria and Trebi was compared including data for all six stations, the latter variety was found to have a hull weight of 0.0041 g per kernel, while the former had a corresponding value of only 0.0036 g. Trebi has larger kernels than Manchuria and this factor more

than offsets the higher percentage of hull when each kernel is considered separately. Barley experts generally recognize that Trebi has a thicker hull than Manchuria.

Averages for the six stations are also presented in Table I. It is apparent that the station where the barley was grown had less effect on the proportion of hull than the variety. This is the chief point of

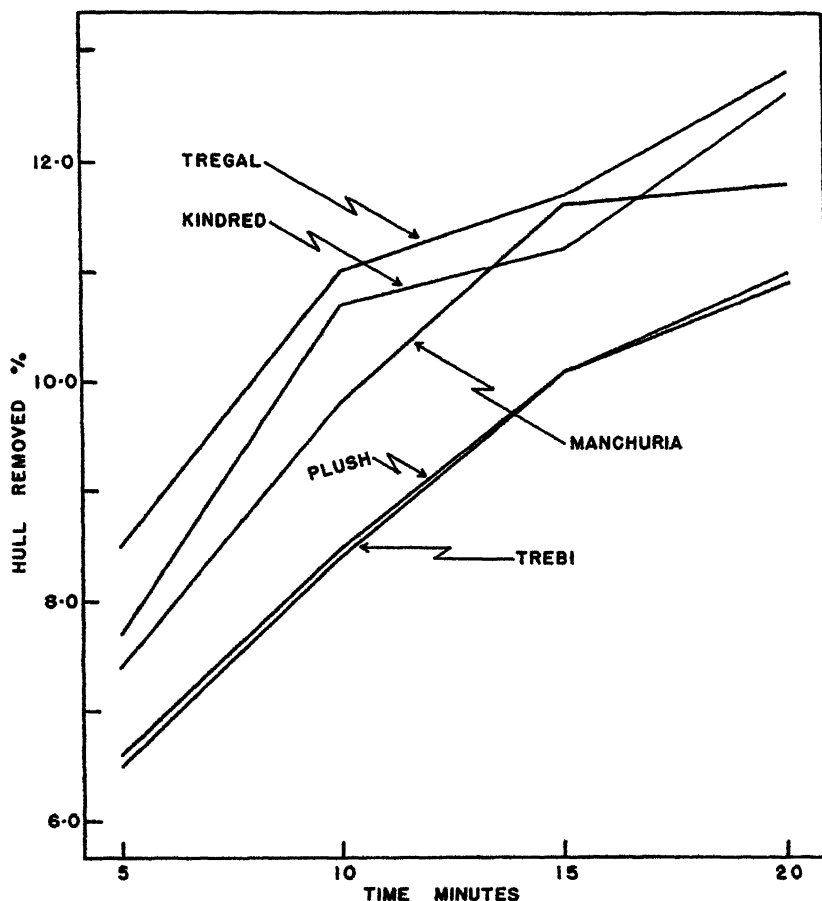


Fig. 3. Relations between percentage of hull removed and dehulling time for five varieties of barley.

interest in the station data since these in general resembled the results from the varieties. The range among stations is only 1.3%, which is approximately the 1% level of significance, while for the varieties it is 2.0%. Little can be stated regarding the effect of the environment in detail on hull percentage, since the environments during seasonal growth at Dickinson and Fargo differ markedly, yet there is no signi-

TABLE II  
ANALYSIS OF VARIANCE OF BARLEY HULL PERCENTAGES

Source of variation	Degrees of freedom	Variance	F
Between varieties	4	19.49	25.64**
Between stations	5	4.52	5.95**
Between times	3	114.11	150.14**
Interactions:			
(Varieties $\times$ stations)	20	1.93	2.53**
(Varieties $\times$ times)	12	0.60	
(Stations $\times$ times)	15	1.67	2.20 <sup>1</sup>
(Varieties $\times$ stations $\times$ times)	60	0.76	
Total	119		

\* Denotes 5% level of significance was attained.

\*\* Denotes 1% level of significance was attained.

ificant difference in hull percentage between the barley grown at these locations. The same is true for the Williston and Edgeley stations.

*Analysis of Variance.* An analysis of variance of the data is shown in Table II. The differences between varieties, stations, and dehulling times were all highly significant with the third source being decidedly the most important, as would be expected from *a priori* consideration. The analysis confirms the findings of Fraser (1944) and the widespread belief that percentage of hull is a varietal characteristic of barley. Obviously this factor should be considered in any barley-breeding program. The environment under which barley is grown also influences the proportion of hull in the kernel; large plump kernels would have a lower proportion of hull than thin or shrivelled kernels. The size of the kernel in any barley is, of course, determined in a large degree by the conditions during growth. The interactions show that the varieties did not all respond in the same manner in respect to the amount of hull when the environment was varied. Changing the length of dehulling treatment did not cause significant variations in amount of hull removed from the varieties. However, the barley grown at different stations did not respond in the same manner to variations in time of dehulling.

*Correlation Coefficients.* Correlation coefficients calculated from the dehulling data for different lengths of time are shown below:

*Variables correlated (figures represent duration of test)*

	$r_{xy}$
5' and 10'	0.665**
5' and 15'	0.524**
5' and 20'	0.178
10' and 15'	0.765**
10' and 20'	0.471*
15' and 20'	0.454*

Note: All correlation coefficients are positive.

\* Denotes significance at 5% point.

\*\* Denotes significance at 1% point.

The value of the coefficient between the five-minute and the other three determinations increases as the time decreases, but the highest value, +0.665, is still too low to permit a precise prediction of hull percent from the five-minute determinations. Nor can results secured from 10 minutes of dehulling be used to predict those attained from 15 minutes. The reasons underlying this lack of agreement between different times of dehulling are not clear at the present time. They may lie in the varying facility with which the hull is removed from different samples of barley. There is, no doubt, some slight abrasion of the kernel itself, although, as pointed out previously, there was little visual evidence of this under magnification (Figure 2). There may also be slight variations in the proportion of the hull remaining in the crease among the various samples. The five-minute run obviously was too short, resulting in incomplete removal of the hull, while the 20-minute determinations were the most apt to result in kernel abrasion due to the lengthy time of exposure of the kernel to bombardment. In addition the latter period was tedious and required too much time. This appears to narrow the choice to the 10- or 15-minute periods for the determination of hull percentage.

The 15-minute period is the best to use because it gives good hull removal with not too much exposure of the kernel to damage. The 10-minute procedure results, however, in fairly satisfactory dehulling and very little opportunity for kernel loss to occur. It is also more economical of time since there is opportunity for weighing out the original samples and reweighing the dehulled samples while the dehulling is being done. These weighings, coupled with recording the data and removing unsuitable material from the original sample and stray unattached hulls from the dehulled barley, rather completely occupy the operator.

The relative rates at which the five varieties relinquished their hulls are shown in Figure 4. These rates, or hull removed per minute, were computed by calculating the ratio  $H/T$ , where  $H$  = percentage of hull removed and  $T$  is time in minutes. It is evident that the rate decreased as the time of dehulling increased. Varietal differences in the rate were most evident at 5 and 10 minutes. It is possible that if other barley varieties were studied, these differences would be more marked. An analysis of variance of the dehulling rates for the four times indicated that significant differences between varieties existed only for the five-minute treatments. The analysis is shown in Table III. This is the time at which the rate of hull removal is most rapid and accordingly the point at which differences in the degree of tightness with which the hull clings to the kernel should be most apparent. As dehulling progresses the rate decreases, as shown in Figure 4.

TABLE III  
ANALYSIS OF VARIANCE OF DEHULLING RATE AT FIVE MINUTES

Source of variation	Degrees of freedom	Variance	F
Between varieties	4	0.16	3.26 <sup>a</sup>
Between stations	5	0.09	1.78
Interaction: Varieties $\times$ stations	20	0.05	
Total	29		

Denotes 5% level of significance was attained.

This would be expected because, as the proportion of hull is progressively removed, the remainder subject to separation will become steadily less, and the rate will decrease.

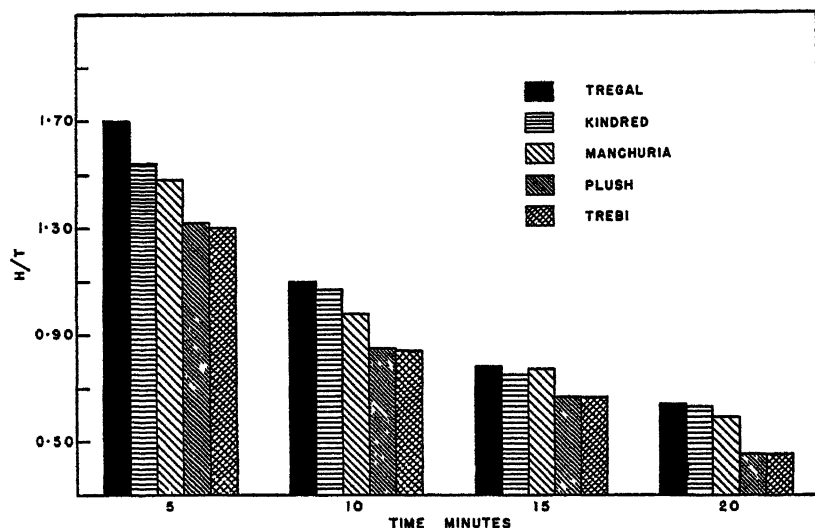


Fig. 4. Comparative rates per minute of hull removal at different lengths of time for five varieties of barley.

### Conclusions

Barley can be readily hulled by the air jet method with a satisfactory degree of reproducibility. No tempering preliminary to hulling is required if the relative humidity is not above 35%, at least for barley grown in North Dakota. This considerably simplifies the procedure. Since the variety-time interaction is nonsignificant, it appears that any time of hulling between 5 and 20 minutes, inclusive, to differentiate varieties in respect to proportion of hull might be

employed. However, if fairly precise hull percentages are required, 15-minute determinations should be employed since the correlation coefficient between any pair of times is too low to permit the use of a prediction equation. If the degree of tightness with which the hull is held is to be ascertained, the five-minute period should be employed. No doubt other seeds can be hulled satisfactorily in this apparatus by employing suitable modifications, as suggested by Fraser.

### Summary

A modification of the air jet method developed by Fraser for determining the proportion of hull in North Dakota barley is described. This method consists of propelling the barley violently against a wire cage lining a metal container. An air jet is employed to propel the barley. Various lengths of treatment were studied and a period of 15 minutes appeared most satisfactory. No preliminary tempering was found necessary.

Very significant differences in hull percentage were found among barley varieties, while location of growth had less effect than variety. Length of hulling treatment had, of course, a very large influence on proportion of hull removed. A significant relation was noted between variety and rate of hull removal only when a five-minute dehulling period was employed. Correlation coefficients between hull removed by different treatments were too low to justify prediction of hull removed by one method from information secured by any of the other three treatments.

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## EFFECT OF PHOSPHATES ON SODIUM CHLORIDE DURING THE ASHING OF SALTED CEREAL PRODUCTS

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An estimation of the original flour ash in flour mixes, breads, and other salted cereal products is frequently needed. Although the official A.O.A.C. method for ashing cereal products, as well as that of Bailey (1937), gives accurate and reproducible results on the original flours, neither of these methods gives reliable results for the original

flour ash in salted products. A number of special methods for this purpose have been proposed but are not entirely satisfactory in that they have all been based on the supposition that sublimation of sodium chloride during incineration is the only mechanism by which loss of this salt occurs.

The purpose of the present study was to disclose, if possible, transformations and reactions of sodium chloride other than direct sublimation during the ashing procedure and to determine which practical conditions of temperature and duration of ashing can be used to give more satisfactory results.

Kalning (1920) proposed a method for determining the original flour ash in bread by the difference between the total ash and the sodium chloride content. His method consists of extraction of the charred bread with acidulated hot water and estimation of sodium chloride in this solution by titration of the chlorides: total ash is determined in a separate operation. The use of finely powdered calcium carbonate to prevent volatilization of sodium chloride during ashing was proposed by Zunino (1934) to permit titration of sodium chloride in the ash. Sgarzi (1934) modified the extraction of sodium chloride by using alkaline solution and recommended the addition of methanol before ashing. A useful but complicated method was introduced by Knottnerus (1916) in which the sodium chloride is extracted with acidulated water and the ash determined on the extracted bread. The sodium chloride is titrated in an aliquot of the extract so that the total sodium chloride in the remainder of the solution can be precipitated by the addition of an exactly equivalent quantity of standard silver nitrate solution. The precipitate is washed and the filtrate plus wash water (containing any sodium in the form of sodium nitrate) is evaporated and the residue incinerated and weighed. The sum of this value and the ash value of the extracted bread minus the sodium oxide equivalent to the total quantity of sodium chloride determined by titration represents the ash content of the original flour.

Comparison of these methods has shown that the results for sodium chloride-free ash are lower than the original flour ash, except in the method of Knottnerus. In addition, the sodium chloride values when determined in the ash are not equivalent to the surplus of the ashes, and the sodium chloride-free ash thus calculated is always higher than expected. This observation suggests that some reaction of sodium chloride beyond its simple sublimation must occur during the ashing procedure. The possibility that sodium chloride may be partially converted to sodium carbonate during ashing was suggested by Pelschenke (1938). The occurrence of sodium carbonate should be detectable by an increase in ash alkalinity. The alkalinity of the ash is

a very erratic value, however, and is not closely correlated to the sodium content of the ash. Moreover, its measurement is difficult and the results obtained are greatly influenced by the method used. It seemed desirable to find some other means of disclosing the reactions of sodium chloride during ashing.

Although the Knottnerus method is capable of giving accurate results, it is cumbersome and time-consuming to an extent unsuitable for extensive series of analyses. This method sidesteps the pitfalls of ashing bread in the presence of sodium chloride but does not explain the necessity for such a procedure. Since it was our purpose to clarify this problem, we used the direct ashing procedure.

### Experimental

The first experiments were designed to measure the amount of sodium chloride that is volatilized and the amount, if any, that is converted to other compounds during ashing. For this purpose, two samples of bread with different salt contents were used. The bread was sliced, dried, ground, and mixed thoroughly. For ashing, 3.0-g. samples were weighed into quartz crucibles. In order to hasten the process, incineration was interrupted three or four times to add a few drops of distilled water. The temperature did not exceed 500°C. Approximately 5 hours were required to complete the incineration and obtain satisfactory light gray ashes.

The sodium chloride was determined in the ash and also in the water extract of the bread samples. For the latter, 5.0-g. samples were washed with three successive 50-ml. portions of boiling distilled water. The extract was cooled and diluted to 250 ml. A 150-ml. aliquot was used for chloride titration according to Volhard. In a similar manner, the ash was extracted with hot water acidulated with a few drops of nitric acid and the chloride titrated as above.

The results obtained for sodium chloride in the bread extract, the ash extract, and the unextracted bread ash as well as the original flour ash are presented in Table I.

It is clear from these data that significantly less sodium chloride was found in the ash extract than in the bread extract. A difference of this amount would hardly be expected to be due to sublimation since the ashing temperature did not exceed 500°C. These data also show that the original flour ash is not reliably indicated by the difference in bread ash and the sodium chloride titrated in the ash extract. The ingredients of bread other than flour and salt influence this relationship, and it seems possible that incompleteness of extraction or the presence of a compound which is not determinable by chloride titration may be involved. The latter is also suggested by the increase



TABLE I

VOLATILIZATION OF SODIUM CHLORIDE UPON ASHING BREAD  
(Results based on 3.0 g. bread)

Bread ash	Flour ash	NaCl in bread extract	NaCl in ash extract	Calc. loss of NaCl in ashing	Bread ash minus NaCl in bread extract	Bread ash minus NaCl in ash extract
mg.	mg.	mg.	mg.	mg.	mg.	mg.
158.7	56.4	117.9	85.9	32.0	40.8	72.8
96.4	56.4	54.3	28.1	26.2	42.1	68.3
121.3	68.7	71.7	29.6	42.1	49.6	91.7
122.5	68.7	71.7	31.8	39.9	49.8	90.7

in the loss of sodium chloride upon ashing with the increase in original flour ash.

Pelshenke (1938) postulated that a reaction of organic compounds with sodium chloride occurs during the ashing process. When pure sucrose was dissolved in salt water and ashed, no evidence of sodium chloride loss was observed. Materials containing metallic impurities, however, caused appreciable loss of sodium chloride upon ashing. Phosphates in particular were observed to be effective in this reaction and were consequently specially studied in view of the phosphates normally used in flour mixes and dough improvers, as well as those contained in the flour itself.

A solution was prepared which contained 0.6% potassium dihydrogen phosphate and 0.25% monocalcium phosphate. When 5 ml. of this solution was evaporated and incinerated several minutes, a residue of 39.6 mg. was obtained. Known amounts of sodium chloride were mixed into the phosphate solution and incinerated 3 minutes in an open Bunsen flame. The residue was cooled and weighed. This process was repeated two or three times, using one minute heating, until constant weight was obtained. The residue was taken up in 5% nitric acid solution and sodium chloride determined by titration as described above. The results of these experiments are presented below:

<i>NaCl in solution</i>	<i>NaCl in residue</i>	<i>Total residue</i>	<i>NaCl-free residue</i>	<i>Blank</i>	<i>Loss of NaCl</i>
mg.	mg.	mg.	mg.	mg.	mg.
60.0	26.3	82.4	56.1	39.6	33.7
60.0	29.1	84.6	55.5	39.6	30.9

These data show clearly that about 50% of the sodium chloride had reacted with the phosphates under these conditions and was no longer titrated as the chloride.

Further experiments were conducted using potassium acid phosphate alone. This compound, as is well known, loses one mole of water when heated and fuses as the metaphosphate ( $\text{KPO}_3$ ). A 0.1 *N* solution of potassium dihydrogen phosphate was prepared and found to yield blanks in good agreement with the theoretical values. Aliquots of this solution to which known quantities of sodium chloride were added were treated as described above. The results obtained are given in Table II.

TABLE II

ASH RESULTS OBTAINED WITH 0.1 *N* POTASSIUM DIHYDROGEN PHOSPHATE CONTAINING KNOWN AMOUNTS OF SODIUM CHLORIDE

Total residue	NaCl in solution	NaCl in residue	NaCl decomposed in terms of		Residue free of sodium oxide and chloride	Blank ( $\text{KPO}_3$ )
			NaCl	$\text{Na}_2\text{O}$		
mg.	mg.	mg	mg.	mg	mg.	mg.
64.6	14.3	0	14.3	7.6	57.0	57.1
135.7	28.6	0	28.6	15.2	120.5	119.0
78.2	28.6	7.9	20.7	11.0	59.3	59.8
79.2	28.6	5.6	23.0	12.2	61.4	60.4
77.1	28.6	2.2	26.4	14.0	60.9	60.4
100.2	57.3	22.8	34.5	18.3	59.1	59.8
101.4	57.3	25.6	31.6	16.8	59.0	59.8
159.4	58.4	14.7	43.7	23.2	121.5	119.0
206.8	116.8	55.5	61.3	32.5	118.8	119.0

These data show that the decrease of chloride in the residue is correlated with the sodium chloride-phosphate ratio. If an excess of phosphate is present during incineration, no titratable chloride can be found in the residue, and apparently under these conditions the chlorine had been quantitatively liberated from the sodium chloride. Losses of sodium chloride were considered here to be the result of a reaction with the phosphate whose end products were sodium oxide and hydrogen chloride. When the amounts of sodium oxide are calculated on this basis, it is evident from the data in Table II that the reaction involved is of this nature, since the calculated values for the sodium chloride-sodium oxide free residues are in close agreement with the blanks.

A study was made of the influence of the sodium chloride-phosphate ratio, as well as of the temperature and duration of incineration, upon the sublimation losses. In the foregoing tests, a dull red temperature was generally reached during the incineration. To increase the amounts of sodium chloride sublimed for this purpose, temperatures of 600°C and higher were used. The data obtained were tabulated as follows:

Period of incineration, minutes	Decrease in weight		
	1 mg.	2 mg.	3 mg.
1.5	30.2	43.2	39.9
1.5	1.5	1.8	3.9
5	0.4	0.8	3.7
5	0.0	0.7	2.7
5	0.0	0.8	2.4
NaCl titr. in residue	0	11.1	55.5
NaCl titr. in solution	29.2	58.4	116.8
NaCl : Phosphate ratio	1/2	1/1	2/1

It is apparent from these data that the largest loss of weight occurs at the start of the heating treatment, and becomes a fairly constant value after a 3-minute incineration period. This is followed by a slow constant loss due to sublimation. It thus appears that disintegration occurs in the first period of heating, when chlorine alone volatilizes, while the intact portion of sodium chloride is subject to sublimation later. No sublimation occurred when the phosphate was in excess, since the initial reaction consumed it entirely with liberation of chlorine. When the sodium chloride is in excess, however, considerable sublimation is to be expected. Practically, sublimation can be avoided only when the sodium chloride-phosphate ratio is held under 1. Thus, it is essential when ashing bread and other salted cereal products in which salt is in excess of this ratio to use as low a temperature as possible and to reduce the duration of incineration by moistening the ash at frequent intervals with a few drops of distilled water or ethanol.

A series of low grade and patent flour samples and a sample of bran to which known amounts of sodium chloride were added were ashed under these conditions to check the reliability of this procedure. The results obtained are given in Table III.

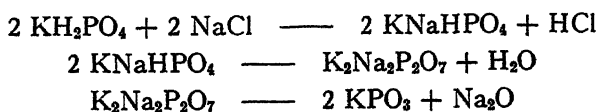
TABLE III  
ASH RESULTS WITH FLOURS CONTAINING VARIABLE AMOUNTS OF  
SODIUM CHLORIDE  
(Results based on 3.0 g. dry matter)

Material	Flour ash	NaCl added	Total ash	NaCl in ash	Na <sub>2</sub> O corresp.	Na <sub>2</sub> O, NaCl free ash
	mg.	mg.	mg.	mg.	mg.	mg.
Low grade flour	48.7	57.2	92.5	29.2	14.9	48.4
Low grade flour	49.0	60.0	95.8	31.6	15.1	49.1
Low grade flour	48.1	60.0	96.4	33.9	13.8	48.7
Low grade flour	48.5	60.0	96.8	34.3	13.6	48.9
Low grade flour	47.0	29.9	62.3	0.5	15.6	46.2
Patent flour	19.1	60.0	73.0	46.2	7.5	19.3
Patent flour	18.0	60.0	68.6	42.3	9.2	17.1
Patent flour	18.8	60.0	66.4	36.2	12.6	17.6
Bran	147.8	60.0	187.2	15.8	23.4	148.0

The calculated sodium oxide and sodium chloride-free ash values are in close agreement with the original ash values and thus show that this procedure can be relied upon for estimation of the original flour ash in salted cereal products where salt is the only ash-bearing ingredient added to the flour.

### Discussion

In view of the effects of phosphates on sodium chloride during the ashing of cereal products, it is apparent that the theories which have been advanced in the past attributing the losses of sodium chloride during ashing wholly to sublimation need modification. Under the usual conditions of ashing, it is suggested that a reaction occurs between the sodium chloride and the phosphates with chlorine being liberated and volatilized and sodium oxide being one of the end products. The reactions may be generalized as follows:



From a practical viewpoint, it is irrelevant whether or not the sodium takes part in the formation of a pyrophosphate molecule, provided the end product is sodium oxide and the calculations are made on the sodium oxide basis. It is probable that the actual reaction is more complicated than the series outlined above and must await further investigation to be fully clarified.

### Summary

The estimation of original flour ash in salted cereal products is unreliable when the ashing is done by means of the common ashing methods.

In the presence of acid phosphates, sodium chloride reacts during incineration, allowing the chlorine to volatilize while the sodium remains in the ash, apparently as the oxide. When phosphates are in sufficient excess, this reaction is very rapid and almost quantitative. Sublimation of sodium chloride only occurs when the sodium chloride-phosphate ratio exceeds 1 at temperatures above 600°C.

The direct ashing method is suitable for estimation of original flour ash in salted cereal products if care is taken to incinerate at temperatures not higher than 500°C and if the phosphate effect is taken into account by computing as sodium oxide the sodium chloride lost during incineration and subtracting from the sodium chloride-free ash value.

### Acknowledgment

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## STUDIES ON BREAD STALING. II. WATER RELATIONSHIPS DURING STALING OF BREAD CRUMB AND THE RETROGRADATION OF STARCH<sup>1,2,3</sup>

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It is commonly observed that, during staling, bread loses its plastic properties, becomes tough, and the crumb loses its ability to swell when added to water (Balland, 1892). It was early shown that this change will occur without any loss of water (Boussingault, 1852). Conversely, it has been shown that if the fresh bread is dried quickly to a low moisture content, the structural evidences of staling fail to develop or do so at a much slower rate (Katz, 1928, 1934; Katz and Weidinger, 1934). These facts indicate that some change in structure is developed in bread during staling which involves or is aided by the water present in the bread. Water of fresh bread acts as a plasticizer, that is, serves as a fluid medium in which motion of the solid particles past one another can take place. It could be that during staling the water becomes

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<sup>2</sup> The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsements of the War Department.

<sup>3</sup> The first paper in this series entitled "Studies on Bread Staling. I. Role of Starch" by T. J. Schoch and Dexter French appeared in *Cereal Chem.* 24: 231-249, 1947.

bound to the solid phase and thereby loses its ability to act as a plasticizing medium in which relative movement of the nonwater particles can occur. If this were true, it should be reflected in the strength and extent of water binding by the solid. If, however, no change in the water binding occurs during staling, it would have to be concluded that the structure which develops must involve the formation of cross linkages between the nonwater elements of the bread. The part played by water in this case would be limited to its function as a medium in which rearrangement of the nonwater components could take place, i.e., in which they could move kinetically to positions relative to each other such as would be required before cross bonding could occur between them.

If, during the course of staling, the water present in the fresh bread should become combined with the nonwater components, it is conceivable that such binding could be either chemical or physical in nature. If chemical binding should occur, such water would no longer exert a vapor pressure and such a process would be reflected in an increase in the dry weight of the bread substance. That no chemical binding of water does occur during staling was indicated experimentally by the fact that the dry weight (after one hour in a forced draft oven at 130°C) of a sample of fresh bread was the same as that of an identical sample of the same bread after it had been allowed to stale while stored in an airtight container for a period of a week at 6°C.

Physical binding of water by nonaqueous materials results only in a decrease in the activity or relative vapor pressure of the water bound. The strength of binding (degree of decrease in activity resulting from the binding) and the extent of binding (amount of water which has been reduced to any chosen activity) may best be estimated in terms of the relative vapor pressure-water content curve per unit weight of the nonaqueous substance under consideration (Briggs, 1932). If the physical binding of the water in bread should change during the staling process, this change would be reflected in the relative vapor pressure-water content curves made on fresh and stale samples of the bread crumb. Should these curves prove to be identical for fresh and for stale crumb, it would be obvious that no change in the water-binding capacity of the nonaqueous components of the bread had occurred, and that the increase in structure must have resulted from cross linkages between the solid elements. If, on the other hand, the relative vapor pressure-water content curve for the stale bread components indicated a large increase in the amount of water bound over that of the fresh bread components, it could be interpreted that the structure developed was due merely to the increased resistance to shear resulting from the decrease in the amount of plasticizer (water)

available as a medium in which relative movement of the nonaqueous constituents could occur. A study of curves for the relation between relative vapor pressure and moisture content of the fresh and stale samples should answer the question as to the possible role of water in the staling process.

In the present studies, relative vapor pressure-water content curves have been obtained on fresh and stale bread crumb prepared from the same loaf of bread and in such a manner as to minimize hysteresis effects other than those resulting from the staling process itself. Similar curves have been obtained on retrograded and nonretrograded samples of whole starch and upon the amylose fraction of starch. Certain conclusions regarding the possible roles played by water and by starch in the staling process are reached on the basis of the experimental findings.

### Methods

Three methods for measuring the relative vapor pressure of the water in bread crumb and starch at various moisture contents were used in this investigation, namely, (a) the isotenoscope technique of Smith and Menzies (1910), (b) equilibration over sulfuric acid solutions of known composition, and (c) a modification of the Brunauer and Emmett (1934) method for measuring the adsorption of a gas on a solid.

The isotenoscope is simply a bulb, into which a sample of moist solid can be introduced, connected through a U-tube to a vacuum pump. An indicator oil is present in the bottom of the U-tube, and a mercury manometer is open to the line beyond the U-tube. In measuring the relative vapor pressure by this method, a sample of the dried, finely powdered solid is moistened with a small amount of water, placed in the isotenoscope bulb which is held in a thermostat at the desired temperature, and subjected to a strong vacuum which causes a part of the water to vaporize and sweep out all other gases from the bulb past the oil in the U-tube. The bulb and its contents are allowed to regain the temperature of the bath, and the vacuum in the line is kept at such a value as to prevent any gas from returning to the bulb. After a short time, during which equilibrium is attained between the adsorbed water in the sample and the water vapor in the bulb, the levels of the oil in the two legs of the U-tube are brought to equal heights and the pressure in the line, which equals the water vapor pressure in the bulb, is read on the manometer. The ratio of this pressure to that of pure water at the temperature of the experiment is the relative vapor pressure of the water in equilibrium with the moist solid. The vacuum is then released, the sample removed into a

weighing bottle which is quickly stoppered, and the wet weight of the sample determined. The sample is then dried for one hour at 130°C in a forced draft oven and the dry weight of the sample found. The difference between the wet and dry weight is a measure of the moisture content in equilibrium with the particular relative vapor pressure determined. The water content per gram of dry sample at that relative vapor pressure is thus a point on the relative vapor pressure-water content curve. The method allows for a quick determination of relative vapor pressures in the range between 0.4–0.8 but is not dependable below 0.4 because of the slowness of attainment of equilibrium, or above 0.8 because of difficulty in obtaining accurate enough pressure measurements in this region where the relation between water content and relative vapor pressure is changing rapidly. Each point to be determined on the curve requires the use of a different sample of solid, which conceivably might introduce some sampling errors.

The second method, that of equilibration of samples of the bread or starch over sulfuric acid solution of known relative vapor pressures, is one which has been used by numerous workers in investigations of this kind. It has the disadvantage that long times (as much as two weeks) are needed for equilibrium to be attained. During this time the temperature must be kept constant in all parts of the closed vessel used to contain the samples and the solution. In earlier studies (Geddes *et al.*, 1946) it was observed that, when the water content of bread crumb was above 20%, staling of the crumb could be detected. For this reason it is doubtful that equilibrium would be attained in this method short of the time required for fresh crumb to change at least partially to stale crumb for samples in which the equilibrium moisture content was greater than 15–20%, and that, therefore, this method could not be depended upon to detect difference between fresh and stale crumb in the relative vapor pressure range lying above this value of water content of the crumb. In the present studies this method was used to supplement the values obtained with the isotenoscope in the R.V.P. range below 0.4.

With a third method, employing a modification of the adsorption apparatus of Brunauer and Emmett, an attempt was made to obtain, on a single sample of the adsorbent (bread or starch), the entire R.V.P.-water content curve for relative vapor pressures varying from 0 to 0.9. Water vapor could be metered into a chamber containing the adsorbent and, after equilibrium had been attained, its pressure determined. Because of many difficulties, not all of which have as yet been overcome, about the only important observation obtained as a result of the use of this method is that, at very low moisture content of the adsorbent (R.V.P. values less than 0.3), very long times



(24 to 36 hours) were required for equilibrium to be attained, while above relative vapor pressures of 0.4, equilibrium was very closely approached in about one-half hour.

### Materials

*Preparation of Bread Samples.* The first part of this study involved the preparation and preserving of bread crumb in relatively fresh and stale states. Farinograph and crumb swelling tests showed that the rate of staling of bread was negligible for bread crumb held below 20% moisture content. The observation of Katz (1928) that freshly baked bread does not stale when held above 60°–70°C was confirmed and this together with the above observation led to a simple procedure for preparing relatively fresh samples of bread crumb. The crumb of freshly baked bread, while still hot, was placed in a vacuum oven and dried overnight at 60°C. In the dry state it did not stale.

Relatively stale bread crumb could be prepared by allowing freshly baked bread to stale in containers at 4°C and then drying the crumb to a low moisture content at room temperature. Under these conditions no reversal of the staling took place.

Both the relatively fresh and stale samples could be stored in desiccators without apparent change for an indefinite period of time.

TABLE I  
EFFECT OF DRYING BREAD ON THE DEGREE OF STALENESS

Sub-sample	Age after baking	Farinograph consistency <sup>1</sup>	Crumb swelling <sup>2</sup>	Crumb moisture
	<i>Days</i>	<i>B U</i>		<i>%</i>
1. Fresh crumb	0.04	585	4.43	44.3
2. Fresh crumb dried at 70°C	1	600	4.16	1.6
3. Fresh crumb dried at 70°C and stored in dry state	8	600	4.02	1.6
4. Stale crumb	6	—	2.88	35.8
5. Stale crumb after drying	6	—	2.91	6.1

<sup>1</sup> Fuller (1938) and Geddes *et al.* (1946).

<sup>2</sup> Katz (1912, 1934a), Cathcart and Lubert (1939), and Geddes *et al.* (1946).

Table I gives some of the results of the work on the preparation of these samples and illustrates that the dried samples so prepared were indeed relatively fresh or stale, respectively, as indicated by crumb swelling and plasticity (Geddes *et al.*, 1946) measurements made subsequent to the drying procedures.

*Preparation of Starch Samples.* The following procedure was employed for the preparation of retrograded and nonretrograded starch, and later for the preparation of corresponding samples of amylose. A 3% starch suspension was gelatinized at 70°C on a water bath and

divided into two portions (one each for the preparation of the retrograded and nonretrograded starch). The portion used for the preparation of nonretrograded starch, while still above 65°C, was transferred immediately to a vacuum oven and dried for 5 hours at 70°C. This material dried to thin, brittle sheets, and it was necessary to grind it to a coarse powder in a Wiley mill before using it in adsorption tests. The nonretrograded starch dried in this way undergoes retrogradation to a much lower extent than when dried at lower temperatures after freezing (see below). As it contained less than 10% moisture, it could be stored in a desiccator for a considerable length of time without change (Katz, 1928). The portion used to prepare retrograded starch was allowed to cool to room temperature and was placed in an icebox at 4°C for 2 to 3 days. This treatment was usually followed by freezing the solution at -10°C. After thawing, the material was dried in air or in a partial vacuum at room temperature. The retrograded starch prepared in this manner contained less than 10% moisture and could be stored in a desiccator.

*Preparation of Amylose Samples.* Starch amyloses were prepared from both potato and wheat starches by the method of Schoch (1942) using n-butanol as the fractionating agent. As recommended by Schoch, the wheat starch was defatted prior to the amylose preparation by refluxing five times with 85% methanol (Schoch, 1942a). The potato starch did not require a similar treatment since its fatty acid content is already extremely low (Schoch, 1942a). The amylose-butanol complexes from the fractionations were stained with iodine and appeared as blue rosettes when viewed under the microscope. The amyloses from the fractionations were washed (with vigorous stirring) three times with methanol, once with ether, and then dried for 2 hours at 65°C in a vacuum oven. This postfractionation vacuum treatment was carried out in order to remove, as completely as possible, any readily volatile nonaqueous substances from the amylose, since these would interfere in the isotenoscope method. The retrograded and nonretrograded amyloses were then prepared in the manner already described for the whole starches.

## Results and Discussions

*Studies on Bread Crumb.* In preliminary trials with the isotenoscope, equilibrium between the moisture adsorbed by bread crumb and its vapor (within the range of R.V.P. values 0.4 to 0.8) was closely approximated within one-half hour. At R.V.P. values below 0.3, equilibrium was not attained even within a 3-hour period. To obtain the R.V.P. values below 0.3, equilibrium measurements were made with the bread crumb exposed over the appropriate sulfuric acid solution.

The first experiment consisted of an investigation of the water-binding capacity of fresh and stale bread crumb. The bread crumb used in this experiment was prepared from fresh white bread baked in the laboratory according to the standard procedure of the A.A.C.C. (*Cereal Laboratory Methods*, 4th ed., 1941). The baking formula contained: 100 g. flour, 5 g. sucrose, 1.25 g. salt, 0.125 g. calcium propionate (to inhibit mold growth), 3 g. yeast, and water as required. The relative vapor pressure–water content curves obtained on these samples are shown in Figure 1.

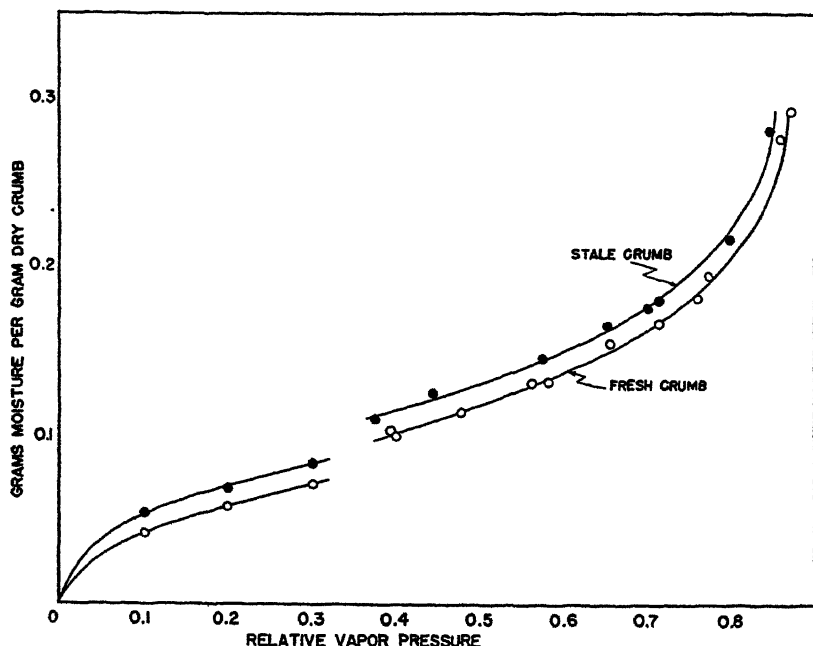


Fig. 1. Relative vapor pressure–moisture content curves for fresh and stale bread crumb. (Isotenscope determinations conducted at 24.6°C. Equilibrium values over sulfuric acid solutions conducted at 25°C and determined after 22 days.)

A discontinuity is apparent in the curves obtained by the two methods. This is undoubtedly due to a failure to attain absolute equilibrium, between moisture deep inside the crumb particle and moisture at the surface, within the 30-minute interval employed in the isotenscope method. The difference in the amount of moisture retained at a given relative vapor pressure by different materials (in this case the difference between moisture retained by stale versus fresh crumb) is, however, nearly the same when determined by either method. Since, in the present research, the difference in water binding is of importance, rather than the absolute amount of water bound, the isotenscope

method is considered to yield results with sufficient accuracy for our purpose, even though these values may not be absolute values. The greater speed of the determination makes this method preferable in the present study, particularly in the higher relative vapor pressure range.

Two observations which are of importance to the problem under investigation are indicated in the curves of Figure 1. The first is to the effect that the differences in water binding by the fresh and stale crumbs are so small as to be insignificant, on the basis of the total water present in bread, as a possible cause of the structural changes which are observed to occur. It can be definitely concluded that these structural changes are not due to a significant decrease in the amount of water which is free to act as a medium of dispersion for the non-aqueous constituents of the bread. The increase in structure during the staling process must, therefore, be due to the formation of some type of cross linkage between the nonaqueous constituents of the bread.

The second point of importance which is indicated by these data is that, while the additional amount of water bound by the stale crumb is very small, it is, nevertheless, definite. It would be expected that, with the formation of cross linkages between the nonaqueous elements of the bread, there should occur a concomitant decrease in the surface area of these components remaining exposed to the water. This should normally result in a *decrease* in the amount of water bound per unit weight of dry bread substances. The fact that the observed change is an increase in water binding during staling suggests the possibility that the cross linkages formed actually occur *through* a water molecule as a part of the bridge or bond between the other components. Such a hypothesis would be strengthened if it could be shown that this observed increase in water binding is proportional to the extent of cross binding taking place in the bread, or bread component, during staling.

Questions which immediately present themselves with respect to this slight but definite increase in water binding resulting from staling of the crumb are: (a) May this increase actually be due to some other conditions of the experiment rather than to a true difference in water binding? (b) What substance of the bread is responsible for this change in water binding, if such it proves to be? (c) Can this slight increase in binding of water result from hysteresis effects other than those associated with the staling process?

This observed difference could conceivably be explained on the assumption that the stale bread crumb had associated with it some nonaqueous substances which were nonvolatile at ordinary temperatures but which might be lost at the temperature of the final drying

during the preparation of the "fresh" bread crumb. The stale crumb had been dried at room temperature only, while the fresh bread crumb had been dried in a vacuum oven at 60°C. Another possibility could be the presence of a greater amount of low molecular weight water-soluble constituents in the fresh crumb than in the stale crumb. Evidence was obtained which showed that neither of these possible factors was operating, and thus the conclusion that stale bread crumb binds a slightly greater amount of moisture than fresh crumb could be drawn with considerable assurance. The methods of obtaining this evidence follow.

To investigate the first of the above possibilities, the stale crumb was treated in a manner which would remove any such nonaqueous volatile substances (if any were present) without causing changes in the degree of staleness of the crumb. This was accomplished by heating a sub-sample of the original lot of stale crumb at 70°C for 5 hours in a forced draft oven. The risk of refreshing the original stale crumb during this process was negligible due to the fact that its moisture content was considerably less than 10% (Katz, 1928, 1934; Katz and Weidinger, 1934; and Geddes *et al.*, 1946). R.V.P.-water content data were obtained for the stale crumb prepared in this manner and, when plotted, all of the points were found to lie on the R.V.P.-water content curve for the original stale sample. This result demonstrated that there were no nonaqueous volatile substances (at least, none that were not also associated with the fresh crumb) which could cause the stale crumb to appear to contain more moisture at any given relative vapor pressure than the fresh crumb.

The second aforementioned condition either did not exist, or did so to such a small extent that it could not be detected, as was indicated by the fact that aqueous extracts of fresh and stale crumb (1 g. of crumb was extracted with 40 ml. of water at 24.65°C for 45 minutes with shaking) gave the same mean freezing point of -0.048°C. This means that the activity of the water present in the fresh and stale crumb was equally depressed by any nonaqueous low molecular weight water-soluble substances which were present.

*Studies on Starch.* It was important to determine if the small observed differences in water-binding capacity of fresh and stale bread crumb were due to some change which the starch, as one important component of bread, had undergone during the staling process. Katz (1912, 1914, 1915, 1928) proposed that the development of crumbliness, increase in crumb rigidity, and decrease in swelling power of the crumb were due to the retrogradation of starch. This hypothesis could be tested by comparing the relative water-binding capacities of retrograded and nonretrograded starch with the corresponding values for

stale and fresh bread crumb This comparison was the first objective of the work on starch.

Water content-R.V.P. curves (Figure 2) were determined by the isotenoscope method for a sample of ungelatinized commercial wheat starch and for retrograded and nonretrograded samples derived from it. The moisture-binding capacity was the greatest for the retro-

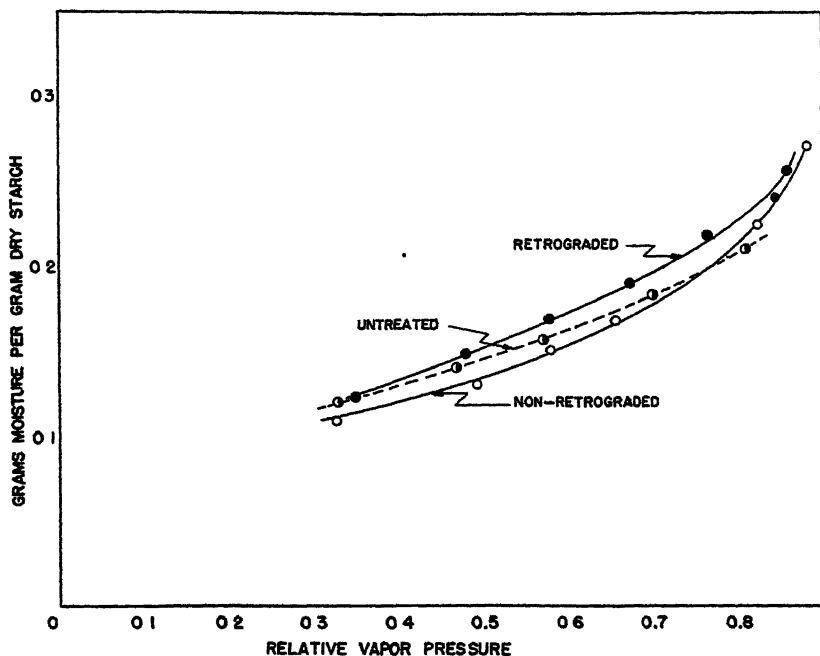


Fig. 2 Relative vapor pressure-moisture content curves for undefatted wheat starch. (Isotenoscope determinations conducted at 24.6°C. Curve 1—retrograded starch, curve 2—untreated starch; and curve 3—nonretrograded but gelatinized starch.)

graded sample and least for the nonretrograded starch, the difference amounting to approximately 0.015 g. of water per gram of dry starch over the range of relative vapor pressures studied. The moisture-binding capacity of the untreated starch was intermediate between that of the derived retrograded and nonretrograded samples. On the assumption that starch in stale bread is retrograded, and that starch of fresh bread is in the nonretrograded condition, the results indicate that the difference in the moisture-binding capacity of stale and fresh bread may be due at least in part to the retrogradation of the starch in the bread.

*Investigations of Possible Hysteresis Effects During Sample Preparations.* The preparations of stale crumb and retrograded starch,

up to this time, had not usually been subjected to a final oven drying previous to the determination of the R.V.P.-water content curves as had been the case with fresh crumb and nonretrograded starch. That the difference observed in water-binding capacities between stale and fresh bread (or between retrograded and nonretrograded starch) was due to the staling (or retrogradation) process alone was indicated by the one experiment on postheated stale crumb already described wherein the postheated and unheated stale crumb gave coincident R.V.P.-water content curves. An investigation of this kind was repeated, however, making certain that hysteresis effects other than those due to staling and retrogradation were held at a minimum. Defatted wheat starch was used in this study.

For this experiment it was necessary to prepare retrograded and nonretrograded starch samples which would have, as nearly as possible, the same previous treatment. The procedure employed was as follows: a 3% starch suspension was gelatinized at 90°C, cooled to 60°C, and divided into two portions. The nonretrograded fraction was prepared from one of the portions (while still at 60°C) by adding a large excess of methanol. The resulting precipitate was collected by vacuum filtration and dried at 55°C at reduced pressure. A saturated solution of sodium bromide in the drying chamber maintained a relative vapor pressure of approximately 0.5 at the drying temperature. The humid atmosphere prevented the starch from drying to a hard, brittle film but allowed it to reach a water content low enough to retard subsequent staling. Retrogradation of the remaining portion of the gelatinized starch was completed after two days' exposure at 4°C. This solution was frozen at -10°C, thawed, and was then added to three volumes of methanol. The purpose of the methanol was to approximate the conditions employed in the preparation of the nonretrograded starch. The precipitated starch was collected on a filter and dried in the manner described above. Treatment with the methanol had reduced its water content to a low enough value so that the subsequent heat vacuum treatment should not cause it to refreshen. Both the retrograded and nonretrograded starches were ground in a Wiley mill (No. 60 screen) and then conditioned to a relative vapor pressure of 0.3 at 25°C.

The relative vapor pressures of the retrograded and nonretrograded defatted whole wheat starches at various moisture contents were determined by the sulfuric acid method at 25°C. The samples were weighed daily after the fourth day. The change in moisture content of the samples was negligible between the seventh and eighth days, and the moisture content became constant by the end of the ninth day.

TABLE II

MOISTURE CONTENT-R.V.P. RELATIONSHIPS IN DEFATTED WHEAT STARCH  
(Tests conducted over sulfuric acid solutions at 25°C)

R V.P.	Moisture per gram of dry starch at the given relative vapor pressure		
	0.1	0.2	0.3
Retrograded defatted wheat starch	g 0.0645	g. 0.0888	g. 0.1050
Nonretrograded defatted wheat starch	0.0595	0.0835	0.1005

These results, given in Table II, show that similar relative relationship exists between the water-binding capacity of the retrograded and nonretrograded starches prepared in the above manner as was observed with the starch and bread crumb samples used in the previous experiments. The observed difference between the water-binding capacity of retrograded and nonretrograded starch is 0.005 g. of water per gram of dry starch. The difference is not as large as would be expected on the basis of the previous data. The discrepancy is undoubtedly due to the differences in the methods of preparing the samples. No independent method was applied to compare the differences in relative degrees of retrogradation between the starch samples prepared by the different methods. This experiment does show, however, that if retrograded and nonretrograded starches have had, as nearly as possible, the same previous history, a slightly greater amount of moisture will still be bound by the retrograded material. This difference thus must be considered as significant.

*Studies on Amylose.* There remained the desirability of obtaining some information concerning the question as to whether or not the observed small increase in water binding which occurs during the staling process in bread and the retrogradation process in starch parallels in amount the degree to which cross linking takes place during the process.

It was reasoned that the degree to which cross linkage could take place, per unit of dry bread or starch substance, before the components were immobilized to such an extent that further formation of such links would be prevented, would be a function of the intimacy with which the components so involved might be able to approach each other. The branched nature of the amylopectin component of starch would tend to prevent it from forming as great a number of cross linkages per unit weight as would the straight-chained amylose component, particularly when the latter was not impeded in the process by the pres-



ence of amylopectin or other components.<sup>4</sup> It was reasoned, therefore, that, per unit mass of solid component, amylose should form a greater number of cross linkages during retrogradation than would whole starch, and whole starch should exceed bread crumb in this regard. If the increase in the binding of water should be proportional to the number of cross linkages formed, then we should find that the difference in water-binding capacity of retrograded and nonretrograded amylose ought to be greater than the corresponding value for retrograded and nonretrograded whole starch. This relationship was therefore investigated.

Measurements made on retrograded and nonretrograded wheat and potato amyloses in the isotenscope demonstrated that retrograded potato amylose bound about 0.035 g. more water per gram of dry amylose than the corresponding nonretrograded amylose. Retrograded wheat amylose bound about 0.025 g. more moisture per gram than the corresponding nonretrograded wheat amylose. As already mentioned retrograded whole wheat starch bound only 0.015 g. more water per gram than nonretrograded wheat starch, and the corresponding figure for stale versus fresh bread crumb was 0.010 g. per gram. It is clearly indicated that the difference in water-binding capacity of stale versus fresh crumb and of retrograded starch versus nonretrograded starch is a function proportional to the degree of retrogradation, i.e., cross linking, which the starch, whether alone or present as a constituent of bread crumb, is able to undergo under the circumstances of the experiment. This evidence, while inconclusive, supports the hypothesis that cross linkages formed during staling of bread and retrogradation of starch are accompanied by a strong fixation of some water molecules in the region of the bond. This could mean that the bond actually occurs through a water molecule or it could mean that where the bond is formed a new region is inaugurated upon which water is strongly adsorbed. That the strength of binding of this water is high, but weaker than a true chemical bond, is indicated by the fact that it will only be removed from the starch at a relative vapor pressure somewhere below 0.05 but above zero R.V.P.

### Conclusions

The role of water in the bread-staling process, insofar as the development of a rigid structure in the bread is concerned, is primarily that of a medium in which rearrangement of starch molecules can occur to positions with respect to one another such that cross linkages

<sup>4</sup> It may well be pointed out, however, that only a few crosslinks between the amylopectin components could result in a greater increase in structure than would many crosslinks involving only the amylose component. The question of importance in this experiment is not that involving the amount of structure but the relative number of cross linkages.

(through hydrogen bonds, presumably) can form between them. The structure generated during staling is due to these cross linkages between nonaqueous elements of the bread and not due to any appreciable increase in the binding of water by these nonaqueous elements.

It appears from these experiments, however, that a very small amount of water is directly involved in the crumb-staling process. The increase in the water bound when bread crumb stales (or when starch retrogrades) is too small (approximately 1-3 molecules for 10 glucose units) to account for the increase in crumb rigidity, on the assumption that water becomes no longer free to act as a plasticizer. Rather, the evidence favors the hypothesis that the increase in crumb rigidity is due to the formation of cross linkages between starch molecules by hydrogen bonds which in some way involve a small number of water molecules. The fact that straight-chained amylose acquires during retrogradation a somewhat higher percentage increase in moisture-binding capacity than whole starch substantiates this point of view.

### Summary

Using an isotenoscope method and a method involving attainment of equilibrium over sulfuric acid solutions of known relative vapor pressures, the relative vapor pressures of water present at various moisture levels in stale and fresh bread crumb, in retrograded and non-retrograded starches, and in retrograded and nonretrograded amyloses were determined.

The relative vapor pressure-moisture content curves, so obtained, indicated that a small but significant increase occurs in the water-binding capacity of stale crumb over that of fresh crumb, and in retrograded over that of nonretrograded starches and amyloses. This increase in water-binding capacity for bread crumb amounts to about 0.01 g. of water per gram of dry crumb. The corresponding figure for whole wheat starch is of the order of 0.015 g., for wheat starch amylose 0.025 g., and for potato starch amylose 0.035 g.

The small increase in water-binding capacity of bread crumb during staling is of no importance as a means of increasing the structural properties of bread through the mechanism of removal of water in its role of a plasticizing medium. The structure developed in staling is due to the formation of cross linkages between the nonaqueous elements of the bread substance. The small amount of water additionally bound during the process indicates, however, that the cross linkages formed may involve some water molecules. The observation that a similar process occurs in the retrogradation of starches and amylose is considered to indicate that these elements are the ones responsible

for the structural evidences of staling in bread crumb. These effects are considered to be in excess to the usual hysteresis effects observable with starch, etc., resulting from simple drying or rehydration processes.

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# GRAIN STORAGE STUDIES. VII. INFLUENCE OF CERTAIN MOLD INHIBITORS ON RESPIRATION OF MOIST WHEAT<sup>1,2</sup>

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The rapid increase in the respiration of seeds at moisture contents in equilibrium with atmospheric relative humidities in excess of about 75%, described by Bailey and Gurjar (1918) and Bailey (1940) as an inherent activity of the seeds themselves, is now known to be due largely to the growth of molds on and in the seed. That molds are the primary cause of heating and deterioration of various kinds of stored seeds at moisture contents where molds can grow has been shown by a number of workers, including Gilman and Barron (1930), Milner and Geddes (1946, 1946a), Milner, Christensen, and Geddes (1947), Nagel and Semeniuk (1947), and Semeniuk, Nagel, and Gilman (1947). In a comprehensive review of the literature on the deterioration of corn in storage, Semeniuk and Gilman (1944) state that "the conditions under which deterioration occurs and the changes which follow its initiation indicate that it is primarily a biological decomposition."

In a preliminary attempt to estimate the inherent respiration of moist wheat independent of that due to molds, Milner (1946) tested 11 sulfonamide compounds as mold inhibitors, and, of these, sulfanilamide proved to be the most effective. In the present studies, sulfanilamide and seven other compounds, to be enumerated later, were tested, the primary aim being to inhibit the growth of molds so that the respiration of the wheat itself could be measured at different moisture contents.

## Materials and Methods

More than 100 compounds<sup>6</sup> were given various preliminary screening tests as fungistatic agents on moist wheat. The wheat was con-

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<sup>6</sup> The following compounds were tested:

acetyl thiourea  
alkyl-dimethyl-benzyl ammonium chlorides  
(Zephiran, or Zephiran chloride)  
alkyl-aryl-sulfonate (Nacconol FSN0)  
alkyl thiourea  
ammonium sulfamate  
p. aminoacetanilide  
4-aminoazobenzene 4-sulfonic acid  
β-amino benzothiazole  
p. aminobenzoic acid  
α-amino butyric acid  
amino guanidine sulfate

1-amino 2-naphthol 4-sulfonic acid  
1-amino 8-naphthol 4,6-disulfonic acid  
p. aminophenol  
o. aminophenol p. sulfonic acid  
2-aminothiazole

benzene sulfonamide  
benzene sulfon-N-dichloramide (Dichloramine B)  
benzoic acid  
borax  
boric acid  
butane monosulfonamide

ditioned to moisture contents between 16% and 25%, then the compounds were, in most cases, applied as dusts, in concentrations of one part by weight of dust to from 100 to 1000 parts by weight of wheat seed. The treated wheat was stored at room temperature in stoppered 250 ml. Erlenmeyer flasks, or in 4 oz. screw-capped medicine bottles, and examined at various intervals up to 30 days. If molds were visible on the treated seed, no further tests were made, but if the grain appeared to be relatively free of molds, 50 to 100 seeds were surface disinfected and placed on nutrient agar to determine the amount of internal mold infection. Similar numbers were placed on agar, moist filter paper, or sterile sand, to determine viability, and a sample was analyzed for fat acidity, which Milner, Christensen, and Geddes (1947) have shown to be a good indicator of mold development.

Of the compounds tested in this way, relatively few prevented visible development of molds on the moist seed. Even some compounds known to be toxic to certain fungi under certain conditions, such as calcium propionate, "chloramine B," "Dithane," the two

calcium propionate  
calcium undecylenate  
carbon tetrachloride  
chlorazene (Chloramine T)  
chloroform  
copper 8-hydroxyquinoline

dichloro-diphenyl-trichloroethane (DDT)  
dichloroethylene  
2,3-dichloronaphthoquinone-1,4 (Naugatuck 604)  
2,4-dichlorophenoxyacetamide  
2,4-dichlorophenoxyacetic acid  
dioctyl ester of sodium succinic acid (Aerosol OT)  
diphenyl  
diphenyl thiosemicarbazide  
diphenyl thiourea  
diphenyl urea  
di-tertiary butyl diperphthalate  
"Dithane A-10" (an experimental product consisting of 85% disodium ethylene, bisdithiocarbamate hexahydrate and 15% of inert ingredients)

epichlorohydrin

hexachlorobenzene  
hexachloroethane  
Hyamine  
hydroquinone  
8-hydroxyquinoline  
8-hydroxyquinoline sulfate  
8-hydroxyquinoline 5-sulfonic acid

isobutylene oxide  
isopropyl glycidyl ether

mercaptobenzothiazole  
methyl guanidine sulfate  
methyl hydrazine sulfate  
2-methyl, 1,4-naphthoquinone

1,4-naphthoquinone

perchloroethylene  
 $\alpha$ -phenoxy propionic acid  
4-phenyl thiosemicarbazide  
phenyl thiourea  
phthalyl sulfathiazole  
propane 1,3-disulfonamide  
propylene glycol  
propylene oxide

quinone

sodium alkyl aryl sulfonate (Nacconol NR)  
sodium anthroquinone  $\beta$ -sulfonate  
sodium benzoate  
sodium N-chlorobenzenesulfonamide (Chloramine B)

sodium propionate  
sucrose

sulfamic acid  
sulfanilic acid  
sulfadiazine  
sulfaguanidine  
sulfamerazine  
sulfamethazine  
sulfanilamide  
sulfapyrazine  
sulfapyridine  
sulfaquinoxaline  
sulfasuxidine  
sulfathiazole  
sulfur

tert. butyl hydroperoxide

tert. butyl perbenzoate  
tetrachloroethylene  
tetramethylthiuramdisulfide  
tetrachloro-para-benzoquinone (Spergon)  
thioacetamide  
thioacetanilide  
thiodiphenylamine  
thiobenzanilide  
thiomalic acid  
thiosemicarbazide  
thiourea  
toluene  
o. toluene sulfonylamide  
p. toluene sulfonylamide  
p. toluene sulfonylmethylamide  
p. toluene sulfonyldimethylamide  
p. toluene sulfonyl n-butylamide  
trichloroethylene  
2,4,5-trichlorophenoxyacetic acid

undecylenic acid  
urea

zinc stearate  
zinc undecylenate

"Nacconols," and "Spergon," did not prevent the visible development of molds on the wheat seed treated with them. In some cases a given fungicide inhibited certain molds, but not others, or inhibited exterior development and spore production of one or more molds, while not inhibiting the growth of molds within the seed. Some of the compounds that inhibited molds also killed the seed.

On the bases of low toxicity to the seed and fair to high toxicity to molds, eight compounds seemed worthy of further testing, namely: 2-aminothiazole, benzene sulfonamide, calcium propionate, chloramine B, 8-hydroxyquinoline sulfate, p-aminobenzoic acid, sulfanilamide, and thiourea. These compounds, as fine powders, were thoroughly mixed into a sound sample of Regent wheat (a hard red spring variety) testing 94% germination. The seed was conditioned to 20% moisture, and the compounds were added at the rate of 1 part per 1000 parts by weight of the damp grain.

In a separate series of experiments thiourea at a concentration of 1.0% of the damp seed weight was dusted on Regent wheat conditioned to various moisture contents from 14.2% to 35.5%. Untreated samples at each moisture were included.

The respiration was measured over a 10-day period with the apparatus and technic described by Milner and Geddes (1945) in which continuously aerated samples of the wheat were held at 30°C and the appropriate relative humidities to maintain the desired moisture contents. The daily accumulations of effluent air were analyzed with a Haldane-Henderson gas analyzer. Care was taken to avoid concentrations of carbon dioxide in the respirometers which might inhibit respiration. Moisture contents using the two-stage, air-oven method, and fat acidity were determined according to the technics described in *Cereal Laboratory Methods* (4th ed., 1941). To determine mold populations at the end of the respiration trials, small lots of seed were ground in an intermediate Wiley mill equipped with a 40-mesh sieve. The mill was first flushed with alcohol to disinfect it and the air-dried samples were ground in succession, beginning with the one of lowest respiratory activity and progressing to the highest. The meals so obtained were cultured at various dilutions in malt-salt agar according to the technic described by Christensen (1946). Germination tests of the seed were made by the Minnesota State Seed Testing Laboratory.

## Results and Discussion

*Evaluation of the Various Fungistatic Agents by Measuring the Respiration of Treated Wheat.* The respiratory rates of the control and of the samples treated with the eight fungistatic agents at a concentration of 0.1% are presented in Figure 1.

The respiratory rates of all the samples, both treated and untreated, were virtually identical during the first two days of the trial, the approximate time required for the molds to begin vigorous growth on wheat at 20% moisture. The shape of the respiration curve for the control sample was essentially similar to a curve of microbiological growth. The effectiveness of the compounds in inhibiting mold growth was roughly inversely proportional to the carbon dioxide production of the treated wheat samples after 7 to 10 days. With thiourea

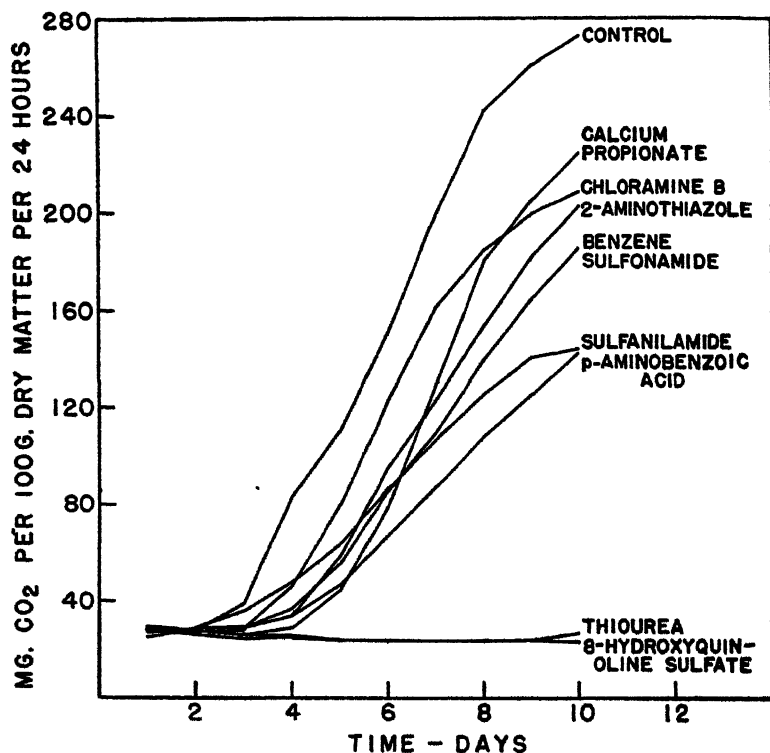


Fig. 1. Influence of time and various mold inhibitors on the respiratory rate of Regent wheat at 30°C containing 20% moisture. The inhibitors were used at a concentration of 1 : 1000.

and 8-hydroxyquinoline sulfate, the respiration remained practically constant (24 to 30 mg. carbon dioxide for 24 hours) for 10 days. This, apparently, approximately represents the natural respiration of this sample of wheat under the conditions of the test.

At the end of the trials, the samples were tested for fat acidity and germination, and the mold population of several samples was determined. These data are presented in Table I, the samples being listed in decreasing order of respiratory rate on the final day of the trial.

TABLE I

INFLUENCE OF VARIOUS FUNGISTATIC AGENTS ON RESPIRATORY RATE, FAT ACIDITY, GERMINATION, AND MOLD GROWTH IN WHEAT CONTAINING 20.0% MOISTURE

(Samples analyzed after completion of 10-day respiration trials at 30°C)

Treatment	Respiratory rate <sup>1</sup>	Fat acidity <sup>2</sup>	Germination <sup>3</sup>	Mold colonies per gram
Control	273.0	77.0	%	6,950,000
Calcium propionate	224.8	91.7	15	4,320,000
Chloramine B	208.6	95.8	21	3,170,000
2-Aminothiazole	202.8	75.3	17	—
Benzene sulfonamide	185.5	52.0	27	—
Sulfanilamide	144.0	65.1	26	2,420,000
p-Aminobenzoic acid	143.7	60.1	49	—
Thiourea	26.3	15.2	92	22,000
8-Hydroxyquinoline sulfate	23.9	16.6	64	—

<sup>1</sup> Mg. CO<sub>2</sub> per 100 g. dry matter per 24 hours on 10th day of trial.

<sup>2</sup> Mg. KOH per 100 g. wheat, dry basis.

<sup>3</sup> Initial germination was 94%.

In general, final fat acidity and mold population decreased with decreased respiration, while the percentage of seed germination increased. The viability of the wheat treated with thiourea was not reduced. Some of the respiration of the sample treated with thiourea was apparently due to molds, since the molds did increase slightly. Although 8-hydroxyquinoline sulfate is an effective mold inhibitor, it was slightly toxic to the seeds.

The data for the daily respiratory quotients of each sample are given in Table II. The course of the respiratory quotient for the control sample was the same as that noted by Milner, Christensen, and

TABLE II

INFLUENCE OF VARIOUS FUNGISTATIC AGENTS ON THE RESPIRATORY QUOTIENT OF WHEAT CONTAINING 20.0% MOISTURE

Respiratory quotient									
Day	Control	Calcium propionate	Chloramine B	2-Aminothiazole	Benzene sulfonamide	Sulfanilamide	p-Aminobenzoic acid	Thiourea	8-hydroxyquinoline sulfate
1	—	0.98	1.14	1.13	—	1.04	—	—	—
2	1.16	1.04	1.05	0.98	0.98	1.03	1.02	1.04	1.08
3	1.03	1.05	1.04	1.02	1.00	1.01	1.06	0.98	1.03
4	1.06	0.99	1.04	1.05	1.09	1.03	1.02	1.01	1.04
5	0.96	0.93	0.99	1.02	1.00	1.03	1.00	1.03	1.03
6	0.88	0.89	0.92	0.95	0.99	0.99	0.98	1.04	1.01
7	0.86	0.91	0.89	0.90	0.94	0.96	0.94	1.05	1.00
8	0.80	0.90	0.88	0.85	0.90	0.93	0.90	1.00	—
9	0.83	0.85	0.86	0.84	0.87	0.88	0.87	1.01	1.04
10	0.81	0.83	0.85	0.82	0.86	0.78	0.86	1.02	1.04



Geddes (1947) for wheat in this moisture range, in that a quotient of about unity in the first few days of the trial was followed by a progressive decrease with increasing mold growth and respiration to a value of about 0.8. The fungistatic efficiency of each of the compounds used was approximately proportional to the extension of the period during which respiratory quotients near unity were maintained. The daily respiratory quotients of the grain treated with thiourea and 8-hydroxyquinoline sulfate remained close to unity throughout the 10-day trial.

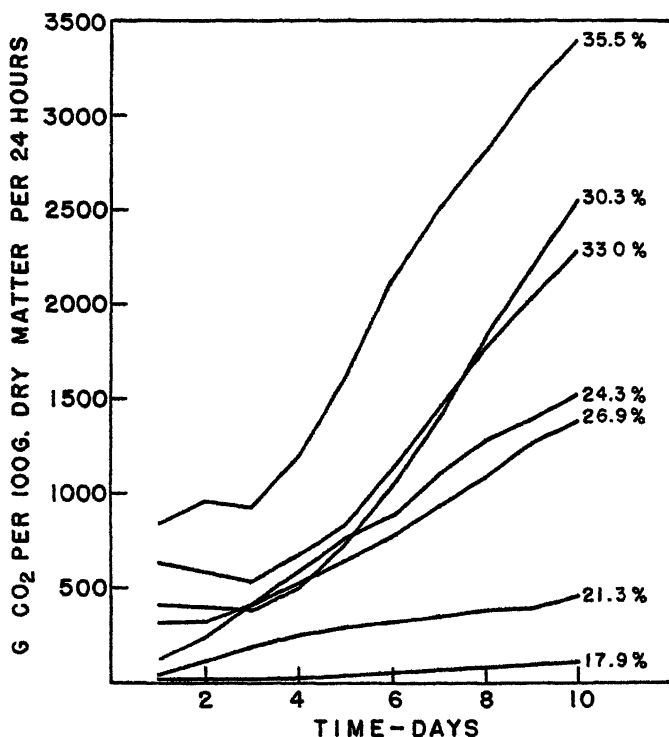


Fig. 2. Influence of time and moisture content on the respiratory rate of Regent wheat at 30°C. Respiratory rates of samples at moisture contents below 17.9% were too low to be indicated on this scale.

*Influence of Moisture Content and Thiourea on Wheat and Mold Respiration.* The fungistatic effectiveness of thiourea as well as its low toxicity to wheat suggested its use for estimating the inherent respiration of wheat at moisture contents up to that required for germination.

Because of the extremely large differences in respiratory rate of the treated and untreated samples, the data of each group are plotted separately. The respiratory behavior of the untreated samples is

shown in Figure 2 and is similar to that described by Milner, Christensen, and Geddes (1947) who showed that increases in respiration due to mold growth occurred in wheat at moisture contents in excess of 14.5%—the equilibrium moisture at 75% relative humidity. The optimum moisture for the growth of different molds varies, and a direct relationship between moisture content and respiratory rate would not be expected, especially at the higher levels where many

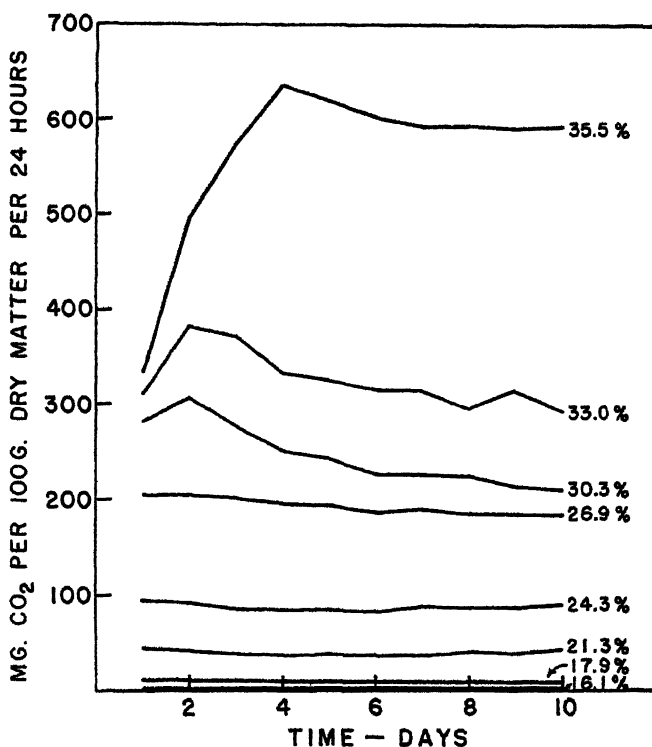


Fig. 3. Influence of time and moisture content on the respiratory rate of Regent wheat at 30°C, treated with powdered thiourea at a concentration of 1%.

species can grow. This may account, at least in part, for the irregularities shown in Figure 2. Thus, after the third day, the wheat at 24.3% moisture had a higher respiratory rate than that at 26.9%; from the fifth to the seventh days the rate for the wheat at 33.0% only slightly exceeded that for the sample at 30.3% moisture after which the rate for the lower-moisture sample became the greater.

The respiratory behavior of the thiourea-treated grain is shown in Figure 3. Except for its property of markedly inhibiting mold growth, and very slightly reducing seed respiration in the first few days, thi-

ourea had no significant influence on the respiratory characteristics of wheat at moisture contents as high as 24.3% and possibly as high as 26.9%, for the seeds respired at very nearly a constant rate throughout the trial. At still higher moisture values (30.3% and 33.0%), however, a brief initial increase in respiration was followed by a steady decrease. The sample containing 35.5% moisture showed a marked initial increase in respiratory activity which lasted for four days, but this trend was then reversed and the respiration declined as the trial progressed. At the end of the experiment many seeds in the sample containing 35.5% moisture had sprouts up to 2.5 cm. in length. Some seeds in the samples with 30.5% and 33.0% moisture also had germinated, a few seeds having sprouts from 0.5 to 1.0 mm. in length. Except for occasional slight swelling of the seed embryos in the grain at 26.9% moisture, no other samples showed evidence of germination.

These observations suggest that the initial increase in respiration shown by samples containing 30.3% and higher moisture, which were treated with thiourea, was due to the germination of the seeds. The subsequent decreases in respiration were apparently due to the death of some of the seeds. The almost linear respiratory values for the samples at moisture values below 26.9% suggest that thiourea is practically nontoxic to dormant seeds in the lower moisture range.

Additional evidence on the influence of thiourea in controlling mold growth and thereby reducing respiration and chemical and germinative deterioration appears in the data of Table III. That thiourea is fungistatic rather than fungicidal to the fungi here encountered is apparent since it caused no decrease in the original mold contamina-

TABLE III

INFLUENCE OF MOISTURE CONTENT AND THIOUREA TREATMENT ON RESPIRATORY RATE, MOLD GROWTH, FAT ACIDITY, AND GERMINATION OF WHEAT  
(Samples analyzed after completion of 10-day respiration trials at 30°C)

Moisture	Respiratory rate <sup>1</sup>		Mold colonies per g.		Fat acidity <sup>2</sup>		Germination <sup>3</sup>	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
%							%	%
14.2	0.16	0.17	5,500	5,766	14.0	18.7	96	97
16.1	2.23	0.94	5,500	4,933	15.9	20.2	94	95
17.9	100.5	6.9	10,166	5,000	50.1	14.1	26	94
21.3	461.2	42.8	5,310,000	23,700	141.2	20.4	11	93
24.3	1512.8	90.6	6,710,000	34,800	92.4	38.5	5	81
26.9	1375.4	184.4	2,580,000	10,660	87.0	21.3	8	19
30.3	2539.4	209.6	65,000,000	77,500	231.4	55.9	—	15
33.0	2267.4	291.8	88,000,000	5,575	222.3	19.2	—	10
35.5	3394.7	592.6	95,000,000	50,000	265.2	58.0	—	2

<sup>1</sup> Mg. CO<sub>2</sub> per 100 g. dry matter per 24 hours on 10th day of trial.

<sup>2</sup> Mg. KOH per 100 g. wheat, dry basis.

<sup>3</sup> Initial germination was 94%.

tion of the seeds but was effective in preventing marked increases. The data do indicate, however, that molds increased slowly in the samples treated with thiourea, at moisture contents of 21.3% and above. The data for fat acidity show that thiourea, through its fungistatic action, inhibited chemical deterioration of the seeds. Seed viability was similarly protected from the toxic effects of mold growth. Viability of the seeds was maintained by thiourea at moisture values

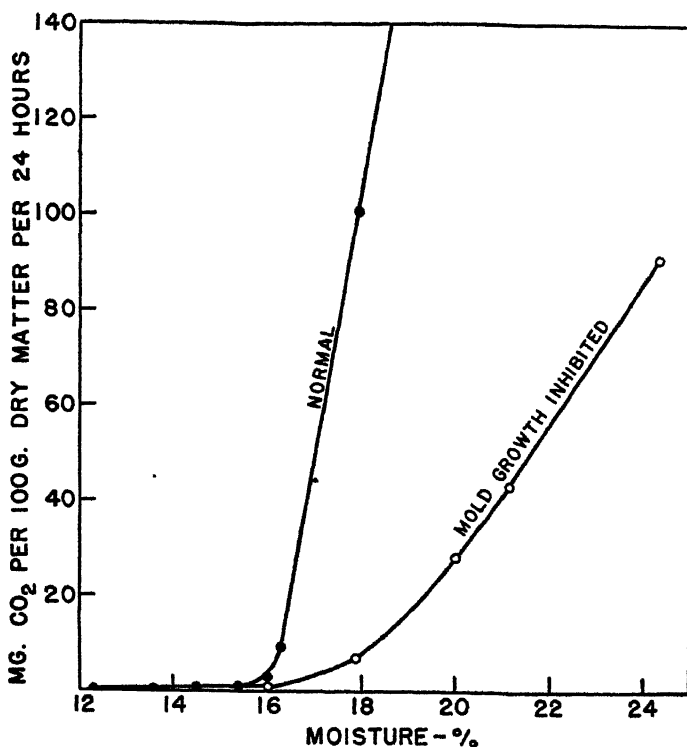


Fig. 4. Influence of moisture content on the respiration of normal wheat subject to mold growth and of the same wheat treated with thiourea at a concentration of 1% to inhibit mold growth, at 30°C. The respiratory trials were conducted for 10 days and the rates plotted for those for the 10th day.

up to and including 21.3%, whereas drastic decreases in germination occurred at moisture contents of 26.9% and above. The minimal moisture at which respiratory anomalies attributable to germination appear (between 24.3% and 26.9%) corresponds to the range of hygroscopic moisture in wheat in equilibrium with 100% relative humidity in the atmosphere at normal temperatures, which has been reported in the literature.

The relation between moisture content up to 24.3% and respiratory rate on the tenth day of the trial for both the normal and thiourea-

treated wheat samples is shown in Figure 4. Additional points on the curve for untreated wheat at moisture contents between 11% and 15% were taken from previous data of Milner, Christensen, and Geddes (1947). The inflection in the moisture-respiration curve for the thiourea-treated wheat occurs at a higher moisture value and is much less abrupt than that for the untreated wheat. The curve for the treated wheat apparently represents principally the influence of moisture content on the inherent respiration of the wheat at moistures up to those at which germinative processes are initiated.

### Summary

More than 100 compounds were tested for fungistatic ability on wheat stored with a moisture content of 16% to 25%. Few of these effectively inhibited the growth of molds on or in the seed. Some compounds inhibited certain molds but not others, or inhibited the surface growth and spore production of certain molds without preventing the growth of the molds in the interior of the seed. This suggests that the effectiveness of a given compound in inhibiting the development of molds on or in moist stored seed of any kind can be ascertained only by determining the number and kinds of molds originally present, and their subsequent increase or decrease after the seed has been treated with the supposed fungicide.

Eight compounds extensively tested as moldicides on wheat with a moisture content of 20% were rated in order of decreasing value as follows: 8-hydroxyquinoline sulfate, thiourea, p-aminobenzoic acid, sulfanilamide, benzene sulfonamide, 2-aminothiazole, chloramine B, and calcium propionate.

Of the two most effective moldicides, thiourea was only slightly toxic to wheat at moisture contents below 24%, while 8-hydroxyquinoline sulfate reduced seed germination more than 30%.

Sound wheat stored at 30°C and at moisture contents above 16.1% was rapidly overgrown by molds. The increase in respiration and decrease in viability of the seed with increasing moisture content was proportional to the increase in molds.

Wheat treated with 1 part of thiourea to 100 parts by weight of moist seed respired at a nearly constant rate over a 10-day period, the viability decreased only slightly, and the molds increased only slightly up to a moisture content of 24.3%, although both molds and respiration had begun to increase at a moisture content of 21.3%. At moisture contents of 26.9% to 35.5% the seed viability was reduced.

The fat acidities of wheat treated with thiourea were markedly lower than those of the untreated grain after storage for 10 days at 30°C, especially at moisture contents above 17.9%.

The respiration of dormant wheat seed on which molds were inhibited (but not eliminated) increased gradually with increasing moisture content until the processes involved in germination became active.

The sharp increase in respiration of wheat at the so-called "critical" moisture content is caused by the respiration of molds on and in the seed.

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## NOTE ON A RAPID METHOD FOR ESTIMATION OF MIXOGRAM AREA<sup>1</sup>

WILLIAM T. YAMAZAKI<sup>2</sup>

(Received for publication August 25, 1947)

Mixogram areas have been used by Morris, Bode, and Heizer (1944) and Lamb (1944) as an index of quality of soft wheat varieties and by Johnson, Shellenberger, and Swanson (1946) to aid in the determination of uses for hard wheat flours. These workers obtained the area by tracing the mixogram with a planimeter which is expensive and rather tedious to use. It seemed likely that mixogram area might be more generally used if a simpler and more rapid method of measuring could be found.

Study of a large number of mixograms, made according to the procedure of Morris *et al.* (1944), revealed that there was a close correlation between area and the sum of two linear measurements; one being the height from the baseline to the center of the band at the point of minimum mobility and the other the height from the baseline to the center of the band at the 7-minute point. Plotting area against this sum showed that the relationship was linear and that most of the points fell close to a regression line.

The method was further tested on 344 individual mixograms of experimentally milled soft wheat flour samples from 11 states representing two crop years and a wide range in varietal quality and protein content (5.7 to 13.8%). The relation of linear measurements to area for the 344 samples is shown in Figure 1. Although the majority of points fall close to a regression line for all samples, there is a considerable number which deviate somewhat. It was found that the samples could be differentiated on the basis of mixing time and the ratio of the two linear measurements. Mixograms exhibiting mixing times of longer than one minute or ratios between height at point of minimum mobility and that at the 7-minute point of less than 1.68 were considered normal. This group included 306 samples.

Further study of the mixograms that were not classed as normal showed that they should be divided into two groups based on the ratio of the height at minimum mobility to that at the 7-minute point, namely those with ratios between 1.68 and 2.00 (34 samples) and those with ratios greater than 2.00 (4 samples).

The statistical data for all samples, as well as for each of the three groups, are as follows:

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

<sup>2</sup> Junior Chemist, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

	Number	Coefficient of correlation	Regression equation
All samples . . . . .	344	0.981	$Y = 0.6132X + 5.462$
Normal samples . . . . .	306	.993	$Y = 0.6345X + 3.555$
Short mixing time samples			
Ratio 1.68-2.00 . . . . .	34	.992	$Y = 0.5765X + 5.717$
Ratio greater than 2.00 . . . . .	4	.995	$Y = 0.5109X + 8.428$

The differences between the correlation coefficients for all samples and those for each of the three groups have been tested and found to be very highly significant. Regression lines for the two special groups

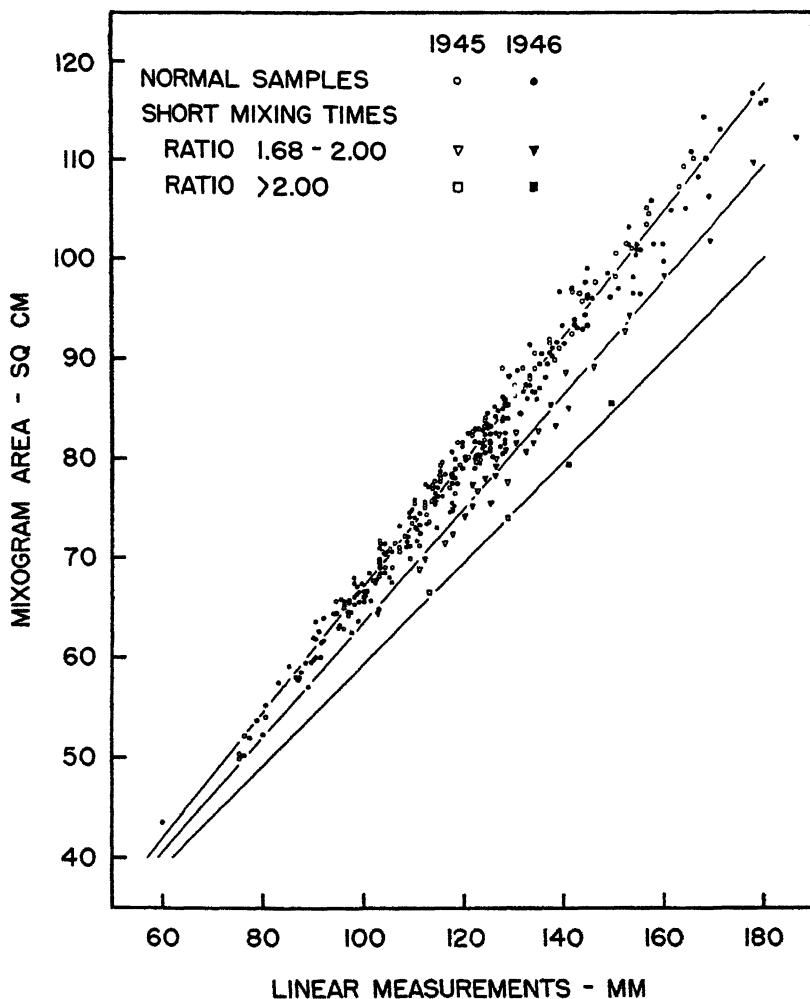


Fig. 1. Mixogram area and sum of linear measurement for 344 samples of soft winter wheat varieties grown at a number of locations and in two crop years.



represent two lower levels and have slopes which are slightly different from that of the normal samples.

The high correlation coefficient for all samples indicates that mixogram area can be predicted accurately from the two linear measurements. In addition, the improved correlation coefficients of the three separate groups compared to that for all samples indicate that by taking into consideration the length of the mixing time and measurement ratio in choosing the regression line, mixogram area can be predicted from the two linear measurements with additional accuracy.

The linear sum (L. S.) may be used as an index of soft wheat flour quality instead of converting it to mixogram area. In the instance of mixograms with short mixing times the linear sum should be adjusted to the same extent as suggested above for areas. This may be accomplished by correcting the linear sum as follows:

for ratio 1.68 – 2.00, corrected L. S. =  $0.9086X + 0.3407$ ,

for ratio greater than 2.00, corrected L. S. =  $0.8052X + 0.7680$ ,

where X = linear sum actually obtained.

If linear measurements are to be used extensively, tables showing corrected L. S. which correspond to various values of X may be prepared from the above equations.

It should be pointed out that, with the exception of Clarkan, relatively few commercial soft winter varieties consistently produce mixograms with mixing times of one minute or less.

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## BOOK REVIEWS

**Methods of Vitamin Assay.** Prepared and edited by the Association of Vitamin Chemists, Inc. 189 pp. Interscience Publishers, Inc., New York, N. Y. 1947. Price \$3.50.

The result of a cooperative effort of some 35 chemists, this volume elucidates the principles and practice of modern nonanimal vitamin assay methods in a thorough and intelligible manner. It is apparent that the majority of the authors have had detailed, firsthand experience with the procedures. Among the 12 methods for the determination of a total of five vitamins and one vitamin precursor will be found examples of virtually all of the basic techniques in current use. These are colorimetric (vitamin A, carotene, ascorbic acid), spectrophotometric (vitamin A), chromatographic (carotene), solvent partition (carotene), fluorometric (thiamine, riboflavin), fermentometric (thiamine), microbiological (riboflavin, niacin), and titrimetric (ascorbic acid).

Individual methods are presented in a detailed fashion not to be found either in the original publications in scientific periodicals, or in the *Official* methods of the U.S.P. and A.O.A.C. The basic principles and the rationale behind many of the precautions are provided and there is a liberal use of very valuable explanatory interpolations. The authors have stressed the applicability of the procedures to specific foods, feedstuffs, and pharmaceuticals. Where necessary, modifications of the extraction procedures, etc., are introduced. In this connection, it is hoped that the reviewer will be pardoned if he alludes to the obvious—technically, *Official* procedures are *official* only when applied to certain specified substances. Thus, the assay of the vitamin content of distillers' solubles, for example, by an *Official* method (U.S.P. or A.O.A.C.) is without any true official standing and, more important, is as likely as not to be in serious error. Therefore, although the procedures in *Methods of Vitamin Assay* are in no sense official, they probably deserve equal rating in many applications and may actually be superior in some instances.

Also emphasized are the experimental pitfalls which lie in wait for the uninitiated or unwary. It is worth mention that current vitamin assay procedures are not simple or easy, except by comparison with the older methods employing experimental animals.

The initial chapter deals with sampling for analysis. It is thoughtfully organized and, following a discussion of general principles, gives detailed consideration to (a) Meats and Other Animal Tissues, (b) Pharmaceuticals, (c) Cereals and Cereal Products and Mixed Feeds, (d) Fruits and Vegetables, and (e) Blood and Urine. The protection of material from the influence of light, heat, and moisture during and after sampling might have received more attention than it does. The preservation of samples for later recheck of the analysis might also be worthy of consideration.

The six succeeding chapters are devoted to the individual vitamins. A selected bibliography of methods for the vitamins not covered in the present volume constitutes the eighth chapter. The ninth and final chapter deals with the use of check samples in the control of vitamin methods and preparations that have been made by the Association of Vitamin Chemists for the distribution of such samples. This is a worth-while service and, if widely used, will improve the quality of both research and routine vitamin assays.

It is to be regretted that the book is relatively limited in scope, covering as it does only five vitamins, but perhaps, as the authors have indicated, this is largely due to the present state of the science. In any case, the present volume can hardly fail to be a very useful addition to the library of any laboratory presently engaged in vitamin assay, or contemplating the same.

In reading the various procedures, this reviewer was reminded of the practice so common during the years in which these methods were being developed. Many of us found that the surest and quickest way to pick up new methods was to visit the laboratory which had pioneered in the particular method and to look over the shoulder of a chemist as he actually made the determination. In a sense, the reader of *Methods of Vitamin Assay* is looking over the shoulder of 35 such chemists. This impression is based on the completeness of the presentation and the attention given to apparently small modifications which, in the words of the authors, are frequently so slight as to seem unworthy of publication, yet, when available, greatly increase the usefulness of the method. Another factor contributing to this impression is the mention, without

prejudice, of specific instruments and sources of reagents that have been found suitable.

The typography and binding of the book are excellent.

LAWRENCE ATKIN  
Wallerstein Laboratories  
New York City

**Modern Cereal Chemistry.** Fourth Edition, by D. W. Kent-Jones and A. J. Amos. 651 pp. The Northern Publishing Co., Ltd., Liverpool, England. 1947. Price \$15.00.

*Modern Cereal Chemistry* has become of recognized value in the chemical literature; consequently, the revisions and additions which appear in the new fourth edition will be welcomed by cereal chemists generally. Dr. A. J. Amos, who contributed much to the third edition, appears as joint author of the present volume.

One of the principal reasons for the popularity of past editions of *Modern Cereal Chemistry* is that it has covered adequately the entire field of cereal chemistry. The new edition continues the policy of presenting the most complete coverage of cereal technology to be found in the English language. The book is particularly valuable because it brings together a combination of selected references to the cereal literature plus the stimulating discussions and evaluations by the authors of controversial topics. The work is much more than a compilation and review of the literature, although in this latter respect it is outstandingly complete. Literature references include articles published during 1946.

The new edition has larger pages than the former edition and space has been conserved by more concise arrangement of material. Thus, although the new book has fewer pages it contains two more chapters than the previous edition. Most of the chapter titles have been reworded slightly, but the subjects covered remain unchanged in general. It is particularly appropriate that one of the new chapters deals with vitamin assay. Barley is now treated in a separate section and a few pertinent facts on soybeans and potatoes are included in the chapter entitled, "Rye, Oats, Maize, Rice, Soya and Potatoes."

Chapter V, entitled "Some Physico-Chemical Aspects of Flour," has been expanded to include a discussion of oxidation-reduction potentials. The theory is presented as well as the significance of  $rH$  values in doughs. Likewise Chapter VI on flour strength brings together in a concise and clear manner the important concepts bearing on the subject. Also, most cereal chemists will likely be pleased by the authors' matter-of-fact discussion of wheat conditioning as presented in Chapter VII. The need for more exact information on wheat conditioning is made evident by the authors.

Cereal chemists on this side of the Atlantic will note with interest that time has brought no change of opinion from our British contemporaries regarding the usefulness of the standard baking test of the American Association of Cereal Chemists. This baking procedure has never been acceptable to European chemists, and for that matter it was probably overpopularized in North America. However, neither are the European baking methods entirely applicable elsewhere; consequently, some of the material in Chapter IX, "The Technique and the Chemistry of the Baking Process," will prove of only cursory interest in North America, but the authors have brought together interesting and stimulating material in this chapter.

As is true of earlier editions, there are chapters on the use of wheat and flour for special purposes and on the nutritive value of cereals. The facts contained in these chapters are readily available through other sources; consequently, their chief value in the book is that the material is at hand in a volume dealing with cereal chemistry. The same argument applies to Chapter XIV, "Cereal and Balanced Rations for Livestock." In contrast, the information presented on the microbiology of cereals is especially valuable because it brings to the attention of cereal chemists facts that are not readily available elsewhere.

The chapter on analytical methods will prove of interest because of the discussions that accompany the procedures and the incorporation of methods not used regularly in North America. Methods for vitamin assay of cereals have been placed in a separate chapter. This should be a convenience.

*Modern Cereal Chemistry* is a book that can be most highly recommended. The authors deserve the thanks of cereal chemists everywhere for preparing a new edition

when much of the work had to be done under the trying conditions that prevailed in England during the war and postwar periods.

However, one cannot but wish that circumstances had permitted the preparation of neater diagrams throughout the book. Also, it is very unfortunate that the material presented in Chapter II, "Principal Wheats of the World," was not brought up to date. Much of the data is no more recent than 1936. In the light of the production changes which the war years caused, there is little justification for devoting space to obsolete crop production data in a book on cereal chemistry.

The book has been carefully printed and the type is easy to read. The publishers have done only a fair job of binding.

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**The Chemical Composition of Foods.** By R. A. McCance and E. M. Widdowson. Second Edition, revised and enlarged. Chemical Publishing Co., Inc., Brooklyn, New York. 1947. Price \$3.75.

This book contains a compilation of analyses of 609 different foods. The tables are given in two separate sections: in the first the composition of foods is presented in terms of grams or milligrams per 100 grams; in the second section in terms of grams or milligrams per ounce. Data are given for water, sugar, starch, total nitrogen, protein, available carbohydrate, calories, sodium, potassium, calcium, magnesium, iron, copper, phosphorus, sulfur, chloride, and acid-base balance. For fruits and vegetables data are also included for unavailable carbohydrate; for meat, poultry, game, and fish, values for "purine nitrogen" are listed. A separate table presents the phytic acid phosphorus content of about 60 foods. No data are given for the vitamin content of the foods listed. With few exceptions the analytical values reported apply to the edible portion of the cooked food; hence they can be applied directly to the calculation of diets. In case of cooked dishes containing several ingredients the recipes for these dishes are given.

A unique feature of these tables of food composition is the fact that they are not assembled from miscellaneous data in the literature; instead they are based on data accumulated in the authors' laboratory over a period of more than 20 years.

In the expression of analytical values and in calculation of caloric value of the foods, the conventions adopted are in some respects different from those commonly used in the American literature. Thus the factor used for the caloric value of carbohydrate is 3.75 because the analytical results are expressed in terms of glucose or of invert sugar; the caloric values for fat and protein are set at 9.3 and 4.1 respectively. The values for acid-base balance of foods are given in terms of  $N/10$  acid or base, while in the American literature they are usually given in terms of  $N/1$  acid or base.

A few random comparisons made between values given by McCance and Widdowson and those of a widely used American source show at times surprising discrepancies; thus the caloric value for butter given in the charts compiled by the H. J. Heinz Company, 12th Edition 1946, is 733 per 100 grams, while McCance and Widdowson give a figure of 793 Calories. Similarly the caloric value for doughnuts is given in these two sources as 425 and 355 respectively. These examples are cited merely to emphasize that the calculation of diets from analytical values in the literature is at best an approximation. For practical dietetics where analysis of the food consumed is usually impossible, such charts as those presented by McCance and Widdowson are of greatest value. *The Chemical Composition of Foods* should be particularly helpful to dietitians, physicians, and home economists.

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**Fatty Acids—Their Chemistry and Physical Properties.** By Klare S. Markley. x + 668 pp., 15 × 23 cm. Interscience Publishers, Inc., New York, N. Y. 1947. Price \$10.00.

As one of a series of monographs on the chemistry and technology of the fats, oils, and related substances (Editorial Board consisting of A. E. Bailey, T. P. Hilditch,

H. E. Longenecker, and K. S. Markley), the purpose of this volume is to provide a comprehensive survey, in an organized and easily accessible form, of the present accumulation of facts and data pertaining to the chemical reactions and physical properties of the saturated, unsaturated, and substituted fatty acids. Particular emphasis is given those acids which naturally occur in the fats and other lipides.

Subject matter, pertinent data, and generally adequate discussions are organized into sections and chapters under headings that aid greatly in making the material readily accessible. An author index (15 pp.) and a subject index (20 pp.) serve to amplify this accessibility. There are 81 graphs and 163 tables of data together with nearly 1500 literature citations. The breadth of coverage is indicated in the following summary of the contents.

A short introductory chapter (11 pp.) on the history and nature of fats and waxes is followed by two chapters (63 pp.) on the classification, nomenclature, and isomerism of the fatty acids. The first major section, which follows, contains five chapters (165 pp.) in which the physical properties of the fatty acids are treated under the headings: crystal properties, spectral properties, thermal properties, solubility of fatty acids and solution properties, and properties of fatty acids in the liquid state. A second major section deals with the chemical reactions of the fatty acids and consists of 11 chapters (277 pp.). Included are extensive discussions on esterification and interesterification, pyrolysis, halogenation, hydrogenation and hydrogenolysis, oxidation and hydroxylation, autooxidation, and the nitrogen derivatives of aliphatic acids, together with short chapters on the salts of fatty acids, alkylation and alkoxylation, biological oxidation, and sulfur derivatives of the fatty acids. The next section of two chapters (43 pp.) is devoted to a fairly comprehensive discussion of the *in vitro* synthesis of the fatty acids and a consideration of the evidences for and against various theories concerned with the biosynthesis of fatty acids. The final section of two chapters (25 pp.) deals with analytical phases of fatty acid chemistry, namely, the separation of fatty acids and the identification of individual fatty acids.

The manner of organization, completeness of treatment, and coverage of subject matter are unique in this volume. It should find wide usefulness among chemists and technologists who are interested in the fatty acids, their products and by-products. Students will find it valuable as a source book of material fundamental to a study of lipide substances.

Few errors, either typographical or factual, were found.

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